

Prevalence of *BRCA1* and *BRCA2* pathogenic variants in a large, unselected breast cancer cohort

Original research

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RUNNING TITLE

BRCA testing for all newly-diagnosed breast cancer patients?

DISCLOSURE OF POTENTIAL CONFLICT OF INTEREST

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ABSTRACT

Breast cancer patients with *BRCA1/2*-driven tumors may benefit from targeted therapy. It is not clear whether current *BRCA* screening guidelines are effective at identifying these patients. The purpose of this study was to evaluate the prevalence of inherited *BRCA1/2* pathogenic variants in a large, clinically representative breast cancer cohort and to estimate the proportion of *BRCA1/2* carriers not detected by selectively screening individuals with the highest probability of being carriers according to current clinical guidelines. The study included 5,122 unselected Swedish breast cancer patients diagnosed from 2001 to 2008. Target sequence enrichment (48.48 Fluidigm Access Arrays) and sequencing were performed (Illumina Hi-Seq 2500 instrument, v4 chemistry). Differences in patient and tumor characteristics of *BRCA1/2* carriers who were already identified as part of clinical *BRCA1/2* testing routines and additional *BRCA1/2* carriers found by sequencing the entire study population were compared using logistic regression models. Ninety-two of 5,099 patients with valid variant calls were identified as *BRCA1/2* carriers by screening all study participants (1.8%). Only 416 study participants (8.2%) were screened as part of clinical practice, but this identified 35 out of 92 carriers (38.0%). Clinically-identified carriers were younger, less likely postmenopausal and more likely to be associated with familiar ovarian cancer compared to the additional carriers identified by screening all patients. More *BRCA2* (34/42, 81.0%) than *BRCA1* carriers (23/50, 46%) were missed by clinical screening. In conclusion, *BRCA1/2* mutation prevalence in unselected breast cancer patients was 1.8%. Six in ten *BRCA* carriers were not detected by selective clinical screening of individuals.

NOVELTY AND IMPACT

This is one of the largest studies on *BRCA1/2* prevalence in an unselected breast cancer population.

INTRODUCTION

Estimates of the prevalence of *BRCA1* or *BRCA2* germline pathogenic variants vary considerably depending on the technology used for mutation screening, population size, and to what extent the genes are tested¹. Although *BRCA1/2* pathogenic variants are major determinants of hereditary breast cancers, women diagnosed with *BRCA1/2*-associated breast cancer do not necessarily exhibit worse survival patterns than breast cancer patients without such pathogenic variants². On the contrary, patients diagnosed with *BRCA1/2*-associated breast cancers have advantages in terms of treatment options when compared to patients with *BRCA1/2* wild-type breast cancer (reviewed in³). Evidence from clinical trials showed significantly greater sensitivity and higher response rate of *BRCA1/2*-associated cancers to neoadjuvant and standard adjuvant chemotherapy than their wild-type *BRCA1/2* counterparts³. Treatment options for *BRCA1/2* breast cancers are also broadened with the introduction of new therapeutic agents, such as poly (ADP-ribose) polymerase (*PARP*) inhibitors, which selectively target *BRCA1/2*-deficient cancer cells⁴⁻⁷.

Recommendation for counselling and genetic screening for *BRCA1/2* pathogenic variants is mainly based on personal and family history of breast and/or ovarian cancer, young age at disease onset, male breast cancer and multiple tumors (bilateral breast cancer or breast and ovarian cancer in the same patient)⁸. However, *BRCA* testing guidelines vary by region and country^{9, 10}. In Sweden, the Swedish Breast Cancer Group *BRCA1* and *BRCA2* screening criteria are used⁸. A report by Nilsson *et al.* estimated that the Swedish *BRCA* testing criteria has an effectiveness of only 18% and concluded that clinical genetic testing criteria for *BRCA1* and *BRCA2* should be critically revised⁸. As the effective identification of *BRCA1/2* germline pathogenic variants has potential to influence treatment decision and has implications for the family of the patients^{3-6, 11, 12}, the pros and cons of testing all women diagnosed with breast cancer for such pathogenic variants need to be examined. In a large, clinically representative breast cancer cohort, we examined the prevalence and characteristics of *BRCA1/2* germline mutation carriers and compared our results with *BRCA* mutation carriers already identified through a national clinical *BRCA* screening program.

METHODS

Study participants

All women under the age of 80 and diagnosed with breast cancer from 2001 to 2008 in Stockholm, Sweden were identified through the Stockholm-Gotland Regional Breast Cancer quality register^{13, 14}. Women were invited to participate in the LIBRO1 study in 2009. In all, 5,715 women of the LIBRO1 study gave informed consent to the retrieval of data from medical records and national registers, answered a detailed questionnaire on background and lifestyle risk factors, and provided a blood specimen for genetic analysis^{13, 14}. Of these women, 5,125 were successfully genotyped in a large-scale genotyping study on breast cancer risk (see **eTable 1 in Data Supplement 1** for exclusion criteria, online only)¹⁵. Of these women, 5,122 had enough DNA remaining for targeted sequencing. The final analytical dataset comprised 5,099 samples which passed quality control. This study was approved by the Regional Ethical Review Board in Stockholm, Sweden (Karolinska Institutet, DNR2009/254-31/4).

Patient characteristics

Self-reported information on education level, age at menarche, body mass index (BMI), number of children, oral contraceptive use, hormone replacement therapy, and details of family history of breast and ovarian cancer were obtained from the questionnaire. Patients were asked if their biological mothers and sisters have been diagnosed with breast or ovarian cancer, and if so, at what age. Mammograms were retrieved from radiology departments. Percent mammographic density was measured using an automated method described in¹⁶. Information on whether the patients have an ovarian cancer or any non-breast malignancy was retrieved via linkage to the Swedish Cancer Register using unique personal identity numbers of study participants (*personnummer*, ten or twelve digit number used in Sweden to identify individuals)¹⁷.

Tumor characteristics

Tumor characteristics were retrieved from the Stockholm-Gotland Regional Breast Cancer Quality Register^{18, 19} using unique personal identity numbers¹⁷. Tumor size was measured in millimetres. Lymph node involvement was dichotomized into positive or negative. Estrogen receptor (ER) status was recorded as negative or positive in the registers, determined by radioimmunoassay or

immunohistochemistry with cutoff values of more than 10% positive cells for IHC and more than 0 fmol/µg DNA for radioimmunoassay assays. The completeness of the registry data was 98% for tumor size and lymph node status and 80% for ER status. Information on grade (Nottingham histologic grade for invasive cancer and nuclear grade for cancer *in situ*) was available from 2004, with 93% completeness¹⁹.

Data on molecular markers were retrieved in 2015–2016 from medical and pathology records at treating hospitals (previously described in²⁰). HER2 status was dichotomized (positive/negative) in accordance with the Swedish Society of Pathology's guidelines: negative if protein expression showed 0 or 1+, or was higher with no confirmed gene amplification by FISH, and positive if FISH showed gene amplification.²⁰ Proliferation marker Ki67 was measured according to contemporary guidelines and reported as percent staining (low if <20% and high otherwise).²⁰ HER2 and Ki67 markers were not assessed, and thus not available in medical records, prior to 2005. Breast cancer subtype was assigned using a random forest algorithm (caret R package, v. 6.0.58) described in²⁰. The algorithm was trained to predict subtype based on a subset of individuals with PAM50 subtype derived from gene expression data (*n*=237). Breast cancer subtype was then assigned to the remaining cases based on age at diagnosis, ER, PR, HER2, and Ki67 status.

Targeted sequencing and data processing

Target-enriched sequencing libraries of germline DNA from 5,122 breast cancer patients were prepared at the Centre for Cancer Genetic Epidemiology (University of Cambridge), as part of a larger effort that included samples from other cohorts. Briefly, target sequence enrichment was performed using 48.48 Fluidigm Access Arrays according to the manufacturer's protocol (Fluidigm, South San Francisco, California, USA). Fluidigm D3 assay design software was used to select primer pairs, which were multiplexed into pools selected for GC content and avoidance of off-target primer-primer and primer-product complementarity (**eTable 2 in Data Supplement 2**). Target sequences were amplified with Illumina sequencing adaptors and one of 1,536 unique sample barcodes (supplied by Fluidigm, South San Francisco, California, USA). Robotic liquid handling and barcode plate identification were used in all steps of the library preparation process. The amplicon library was quantified with the KAPA Library Quantification Kit (KapaBiosystems, Boston, Massachusetts, USA) and then sequenced on the Illumina Hi-Seq 2500 instrument using v4 chemistry, according to the

manufacturer's protocol (Illumina, San Diego, California, USA). Each library was sequenced 2-3 times to provide sufficient coverage. Details on sequence data processing and quality control are shown in **eMethods** in **Data Supplement 1**. A total of 5,099 samples had valid variant calls. The mean read depth across the coding sequences of *BRCA1* and *BRCA2* was 792.2 (standard deviation: 587.4) and 631 (standard deviation: 516), respectively. More than 90% of targeted bases had more than 15x coverage (94.8 [15.9] and 92.5 [20.4] for *BRCA1* and *BRCA2*, respectively).

Definition of pathogenic variants

As described previously in Borg *et al.*²¹, sequence variants were categorized based on their predicted effect on the mRNA and amino acid level and defined as pathogenic if they were (1) frameshift and nonsense variants with the exception of the *BRCA2* c.9976A>T (BIC: K3326X) and other variants located 3' thereof ($n=105$), and (2) all consensus splice acceptor or donor sequence sites, except those predicted to lead to naturally occurring in-frame RNA isoforms that may rescue gene function²². Public data on pathogenic *BRCA* variants (includes frameshift insertion/deletions, nonsense, splice sites and missense variants conclusively demonstrated to be pathogenic) that have been curated and classified by an international expert panel, the ENIGMA consortium, were also downloaded from <http://brcaexchange.org/> (access date: Feb 22, 2017) for the annotation of the sequence data.

Identification of women who have undergone *BRCA* testing in Sweden

Mutation screening for all oncogenetic clinics in Sweden (Lund, Stockholm, Uppsala, Göteborg, Linköping and Umeå) were conducted at the Department of Oncology, Lund University as part of a national *BRCA* testing program (**eMethods** in **Data Supplement 1**). We cross-referenced the personal identity numbers of all study participants in LIBRO1 with the *BRCA* testing unit at Lund University to identify women who have been tested for *BRCA1/2* pathogenic variants previously. The SweBRCA criteria are the only *BRCA1/2* testing criteria used in Sweden (**eTable 3** in **Data Supplement 1**)⁸. Clinicians do not have any obligation to comply with the guidelines⁸.

Statistical analysis

Predictor variables which include patient and tumor characteristics were described by the counts of each category and corresponding proportions. Binary logistic regression models were fitted

for the dichotomous outcome (*BRCA1* [reference] and *BRCA2*), and multinomial logistic regression models were fitted for the three-category outcome (*BRCA1*, *BRCA2* and non-*BRCA* [reference category]), adjusting for age and year of diagnosis. Logistic regression models were also used to compare estimates (odds ratios [OR] and corresponding 95% confidence intervals [CI]) of patient and tumor characteristics between *BRCA1/2* carriers already identified among a subset of 416 patients screened as part of clinical *BRCA* testing routines and additional *BRCA1/2* carriers found by sequencing the entire study population (i.e. those not tested by the Swedish *BRCA* testing program).

RESULTS

The median time from date of diagnosis to study entry is 4.8 years (range: 1.3 to 9.2). The median age of breast cancer diagnosis of the study cohort was 59.6 years (range: 25.1 to 79.9). Nine of ten breast cancers were invasive (89.4%).

Spectrum of *BRCA1* and *BRCA2* pathogenic variants

Of the 5,099 breast cancer patients, 92 (1.8%) were identified as *BRCA1/2* carriers (50 *BRCA1* carriers and 42 *BRCA2* carriers) and 5,007 were non-*BRCA*.

Among the 50 *BRCA1* carriers, there were 28 unique germline *BRCA1* pathogenic variants (11 frameshift deletions, 2 frameshift insertions, 8 truncating, 4 splice sites, and 3 missense) (**Figure 1** and **eTable 4 in Data Supplement 1**). Frameshift insertions and deletions made up 26/50 (52%) of the *BRCA1* pathogenic variants. Exon 11 harbored 33/50 (66%) of the *BRCA1* pathogenic variants. The most common pathogenic variant was c.3048_3052dupTGAGA ($n=8$), which is a founder mutation originating from the West coast of Sweden²³. Three other Swedish founder pathogenic variants were also identified (c.1082_1092del [$n=5$], c.2475delC [$n=2$] and c.3626delT [$n=3$])²³⁻²⁶.

Among the 42 *BRCA2* carriers, there were 33 unique *BRCA2* pathogenic variants (18 frameshift deletions, 3 frameshift insertions, 9 truncating, and 3 splice sites) (**Figure 2** and **eTable 5 in Data Supplement 1**, only online). Over half of all *BRCA2* carriers (24/42, 57.1%) had a pathogenic variant on exon 11.

Patient characteristics of non-*BRCA*, *BRCA1* and *BRCA2* carriers

Half of the non-*BRCA* women were at least 60 years old, compared to 26.0% and 33.3% for women with *BRCA1* and *BRCA2* pathogenic variants, respectively (**eTable 6 in Data Supplement 1**).

In the crude analyses controlling for age and year of diagnosis, *BRCA1* and *BRCA2* carriers were more likely than non-*BRCA* women to report family history of both breast ($OR_{BRCA1 \text{ vs non-}BRCA}$: 4.00 [2.27 to 7.05] and $OR_{BRCA2 \text{ vs non-}BRCA}$: 2.23 [1.17 to 4.26]) and family history of ovarian cancer ($OR_{BRCA1 \text{ vs non-}BRCA}$: 7.53 [3.82 to 14.82] and $OR_{BRCA2 \text{ vs non-}BRCA}$: 3.62 [1.50 to 8.71]) (**eTable 6 in Data Supplement 1**). *BRCA1* carriers, in particular, were also more likely to be also diagnosed with an ovarian cancer themselves ($OR_{BRCA1 \text{ vs non-}BRCA}$: 28.02 [10.72 to 73.29] and $OR_{BRCA2 \text{ vs non-}BRCA}$: 8.11 [1.87 to 35.24]) than non-*BRCA* patients (**eTable 6 in Data Supplement 1**). *BRCA1* carriers were more likely to have a personal history of another malignant cancer in addition to their breast cancer than patients with non-*BRCA* patients ($OR_{BRCA1 \text{ vs non-}BRCA}$: 2.93 [1.37 to 6.27]). This association was driven by ovarian cancers ($OR_{BRCA1 \text{ vs non-}BRCA}$ for all non-breast and non-ovarian malignancies: 0.83 [0.25 to 2.73]). *BRCA2* carriers were significantly less likely to be ever users of hormone replacement therapy (HRT) than non-*BRCA* breast cancer patients (26.2% vs 53.8%) (**eTable 6 in Data Supplement 1**). In multivariable models shown in **Table 1**, all variables remained significantly associated, with the exception of personal history of any non-breast malignancy.

Tumor characteristics of *non-BRCA*, *BRCA1* and *BRCA2* carriers

In the crude analyses controlling for age and year of diagnosis, *BRCA2* carriers were in general not significantly different from non-*BRCA* women in terms of tumor characteristics, with the exception of nodal involvement ($OR_{BRCA2 \text{ vs non-}BRCA}$: 2.71 [1.31 to 5.62], **eTable 7 in Data Supplement 1**). On the contrary, tumors of *BRCA1* carriers were more aggressive than those of non-*BRCA* breast cancer patients for all tumor characteristics examined (ER and PR status, grade, tumor size, nodal involvement, and breast cancer subtype) except for the proportion of invasive tumors (**eTable 7 in Data Supplement 1**).

In multivariable multinomial models including all tumor characteristics that were significantly different between non-*BRCA* and *BRCA1*-positive breast cancer patients, only ER-negativity remained significant ($OR_{BRCA1 \text{ vs non-}BRCA}$: 5.19 [2.68 to 10.06]) (**Table 1**). ER status was also the only independent tumor characteristic that distinguished between *BRCA1* and *BRCA2* carriers ($OR_{BRCA2 \text{ vs BRCA1}}$: 0.22 [0.07 to 0.77]). This observation was mirrored in a separate multinomial model considering breast cancer subtypes, where *BRCA1* tumors were found to be 40 times more likely to be of the basal-like subtype ($OR_{BRCA1 \text{ vs non-}BRCA}$: 40.07 [14.26 to 112.59]). Only nodal involvement remained

significant in the comparison between *BRCA2* and non-*BRCA* breast cancer cases in the multivariable model ($OR_{BRCA2 \text{ vs non-}BRCA}$: 2.54 [1.20 to 5.37]) (**Table 1**).

Comparison of *BRCA1/2* carriers identified versus not identified through clinical screening

Linkage with the Swedish *BRCA* register found 416 patients (8.2%) that were screened for pathogenic variants as part of routine clinical practice. Among these 416 women, clinical screening identified 39 carriers in the study cohort, of which our study confirmed 35 (**Figure 3**). Four pathogenic variants were missed (*BRCA1*: c.4186-1785_4358-1667dup and c.4358-1729_4986+736dup; *BRCA2*: c.7805+1538_8331+560del and c.9097_9098insT) (**Figure 3**). Three of these were large exonic deletions or duplications that the Fluidigm Access Array system is not suitable for detecting. This gives the Fluidigm Access Array method an estimated sensitivity of about 90%, or 97% when excluding large exonic variants.

Overall, 57/92 carriers (62.0%) were not already clinically identified: Two additional carriers were detected by the Fluidigm Access Array method among clinically screened patients (*BRCA2*: c.2578delA [confirmed by Sanger sequencing to be a false positive] and c.7443delT [missed carrier, screened with DHPLC and MLPA in 2008]); the remaining 55 out of 92 carriers (59.8%) identified by the Fluidigm Access Array method in the complete study cohort were never screened as part of clinical routine (**Figure 3**).

More *BRCA2* (34/42, 80%) than *BRCA1* pathogenic variants (23/50, 46%) were missed by selectively testing only high-risk individuals who were recommended for genetic testing and counselling (**Table 2**). Controlling for only year of diagnosis, *BRCA* carriers identified by clinical routine screening were younger (37.2% aged 50 years and above, compared to 73.7%), less likely to have experienced menopause ($OR_{\text{identified versus not identified}}$: 0.17 [0.07 to 0.44]) and more likely to be associated with a family history of ovarian cancer ($OR_{\text{identified versus not identified}}$: 3.11 [1.06 to 9.09]) (**Table 2**). Further adjustment for gene revealed a significant association with age at menarche ($OR_{\text{identified versus not identified}}$: 2.99 [1.00 to 8.94]). There was also a trend between the likelihood of being identified as a carrier by selective testing and more children (**Table 2**). Tumors of *BRCA1/2* carriers identified by selective testing were more often detected clinically ($OR_{\text{identified versus not identified}}$: 5.52 [1.38 to 22.18]), higher grade ($OR_{\text{identified versus not identified}}$: 0.28 [0.08 to 0.92]), larger size ($OR_{\text{identified versus not identified}}$: 2.48 [1.00 to 6.16]) and of a basal subtype ($OR_{\text{identified versus not identified}}$: 6.07 [1.49 to 24.76]) (**eTable 8 in Data**

Supplement 1). The differences observed for all tumor characteristics and selective testing detection did not remain significant after adjusting for gene.

DISCUSSION

BRCA1/2 pathogenic variants were found in 1.8% of unselected breast cancer patients. In contrast to studies reporting *BRCA1/2* prevalence for a subset of high risk women^{27, 28}, the present sample reflects the general breast cancer population. None of the breast cancer risk factors examined differed between *BRCA1* and *BRCA2* carriers. However, *BRCA1* and *BRCA2* breast cancers differed in the proportions of patients with ER-negative disease and basal-like subtype. Six out of ten *BRCA1/2* carriers were not identified through genetic testing in the clinic.

BRCA1 and *BRCA2* mutation frequencies in breast and ovarian cancer patients unselected for family history or age at onset are generally low (<1–7% for *BRCA1* and 1–3% for *BRCA2*)²⁹. The combined *BRCA1/2* mutation frequency in a Swedish population of unselected breast cancer cases recruited from 1998 through 2000 in Stockholm has been previously estimated to be not more than 1% in the work by Margolin *et al.*¹. In that study, screening for *BRCA1* pathogenic variants was limited to exon 11, which covers over half the coding region of *BRCA1*³⁰. More than 70% of diagnosed pathogenic variants including four founder pathogenic variants in the Swedish population are known to be located on this exon^{31–33}. Prevalence of *BRCA2* pathogenic variants in the Swedish population was deemed by Margolin *et al.* to be negligible among unselected breast cancer patients due to the low frequency of such pathogenic variants even in high-risk groups in the region¹. On the contrary, only 33 of 50 *BRCA1* pathogenic variants were identified on exon 11 in this study, thus suggesting that 34% of *BRCA1* carriers would have been missed if exon 11 alone were screened. Through testing the entire sequences of *BRCA1/2* genes with improved methodology and techniques, we estimate the combined prevalence of *BRCA1/2* pathogenic variants among unselected breast cancer patients in Sweden to be closer to 2%.

There are close to 2,000 known *BRCA1* germline pathogenic variants, many of which are loss-of-function frameshift pathogenic variants³⁴. Nine of 28 (32%) unique *BRCA1* and 6 of 33 (18%) unique *BRCA2* pathogenic variants were found to be recurrent in Swedish breast cancer patients (i.e. pathogenic variants that were found to occur in at least two unrelated individuals). The relatively low recurrent mutation frequency, including that of Swedish founder pathogenic variants, would mean that

screening of selected pathogenic variants alone may not be a sensitive approach in this population as majority of *BRCA1* and *BRCA2* carriers will have been missed. While *BRCA1* pathogenic variants confer a more aggressive tumor phenotype, *BRCA2* pathogenic variants typically resemble sporadic breast cancer³⁵. There is good agreement between our observed results regarding the tumor characteristic differences between *BRCA1/2* and non-*BRCA* breast cancer cases and what has been previously reported in literature. It has been observed by others that tumors in *BRCA1* carriers more frequently exhibited high mitotic count, high grade, ER and PR negativity³⁶⁻³⁸. A large proportion of *BRCA1* mutation cases (~80%) have also been documented to be triple negative and basal-like breast cancers³⁶⁻³⁸. In a Swedish study where 54 female breast cancer patients from 22 families with *BRCA2* germ line pathogenic variants from Sweden and Denmark were compared with 214 age- and date of diagnosis-matched controls identified among breast cancer patients from South Sweden, *BRCA2*-associated cases were more often node-positive than non-*BRCA* cases³⁹. Other than nodal involvement, *BRCA2*-associated breast carcinomas were generally associated with less aggressive tumor characteristics than *BRCA1* cancers, and were more likely to be hormone-related^{37, 38}.

Thirty-eight percent of *BRCA1/2* carriers were identified through selective clinical testing of 8.2% of breast cancer patients. Grindedal *et al.* evaluated the results of *BRCA1/2* testing in South-Eastern Norway and found that 65% of the *BRCA1/2* carriers would have been missed if using age of onset below 40 or triple negative breast cancer as criteria for testing⁴⁰. It is also conceivable that, due to an emphasis on disease family history in current guidelines, a smaller family size may compromise the identification of high risk individuals who would otherwise benefit from genetic testing⁴¹. In a Swedish retrospective study by Nilsson *et al.* where all breast cancer patients were tested, it was found that while 65% of the *BRCA1/2* carriers fulfilled Swedish criteria for testing, only 18% had been identified in regular clinical routine⁸. Other factors such as varying compliance with guidelines for the recommendation of *BRCA* testing by clinicians will lead to even more *BRCA1/2* carriers being missed. It may thus be of benefit to test all newly diagnosed breast cancers in light of available targeted therapy options.

To our knowledge, this is the largest population-based breast cancer testing study for *BRCA1/2* published outside of founder populations. Despite the richness of the data which encompasses patient and tumor, some risk groups were too small to be examined with adequate statistical power (e.g. benign breast disease). The Swedish health care system is mainly government-

funded and decentralized, making it possible to identify all women who went for clinical *BRCA* testing. Nonetheless, private health care also exists, and some *BRCA1/2* carriers may have been identified by commercial testing outside the public sector. However, the number of patients tested outside of the national *BRCA* testing program is likely negligible during the period 2001-2008⁸. It should be also noted that the Fluidigm Access Array method used cannot detect large rearrangements and has a sensitivity of ~90%, hence further analytical validity studies are needed. More sensitive methods and the universal *BRCA* testing of newly breast cancer patients will help to increase the number of women getting the best treatment for their disease.

In summary, *BRCA1/2* pathogenic variants were found in 1.8% of an unselected Swedish breast cancer cohort. Six out of ten *BRCA* carriers were not identified through selective clinical testing routines. Our results give fruitful information for further decisions of *BRCA* testing for all breast cancer patients at time of diagnosis. The presented data can be a starting point for further studies dealing with issues such as cost effectiveness of screening patients with different tumor characteristics and patient health attitudes.

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FIGURE LEGENDS

Figure 1. Mutation plots of *BRCA1*. Four and three splice variants for *BRCA1* (NM_007294.3) are not shown.

Figure 2. Mutation plots of *BRCA2*. Three splice variants for *BRCA2* (NM_000059.3) are not shown.

Figure 3. Overlap between women attending *BRCA* screening (clinically tested), *BRCA* carriers identified through selective clinical testing routine (clinically-detected carriers), and *BRCA* carriers identified through screening all unselected LIBRO1 breast cancer patients (unselected-detected). Of the 416 women who were clinically tested, 39 were found to be *BRCA1/2* carriers (39/416, 9.3%). Our study confirmed 35 of these pathogenic variants. Four pathogenic variants were missed (*BRCA1*: c.4186-1785_4358-1667dup and c.4358-1729_4986+736dup; *BRCA2*: c.7805+1538_8331+560del and c.9097_9098insT). By sequencing the entire Swedish study, we found 55 more carriers who were not screened as part of clinical routine.

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Table 1. Odds ratio (OR) and corresponding 95% confidence intervals (CI) of predictors according to *BRCA* status.

	<i>BRCA1 vs non-BRCA</i> OR (95% CI)	<i>BRCA2 vs non-BRCA</i> OR (95% CI)	<i>BRCA2 vs BRCA1</i> OR (95% CI)
<i>Model 1: Patient characteristics</i>			
Age at diagnosis: 50-59	0.21 (0.10 to 0.45)	0.78 (0.36 to 1.69)	3.55 (1.05 to 11.97)
Age at diagnosis: ≥ 60	0.14 (0.06 to 0.31)	0.55 (0.24 to 1.23)	3.91 (1.11 to 13.84)
Year of diagnosis: 2005-2008	1.68 (0.91 to 3.08)	1.03 (0.55 to 1.92)	0.90 (0.33 to 2.48)
HRT ever: Yes	1.08 (0.56 to 2.10)	0.36 (0.17 to 0.76)	0.31 (0.10 to 0.93)
Family history of breast cancer: Yes	3.57 (1.99 to 6.41)	2.08 (1.08 to 3.99)	0.60 (0.24 to 1.55)
Family history of ovarian cancer: Yes	6.99 (3.43 to 14.24)	3.57 (1.47 to 8.68)	0.38 (0.11 to 1.35)
Personal history of ovarian cancer: Yes	19.21 (5.89 to 62.72)	8.01 (1.61 to 39.94)	0.49 (0.04 to 6.74)
Personal history of any malignant cancer (not breast): Yes	1.35 (0.52 to 3.54)	0.81 (0.26 to 2.56)	0.49 (0.07 to 3.59)
<i>Model 2: Tumor characteristics, adjusted for age and year of diagnosis</i>			
Detection mode: Interval	1.34 (0.38 to 4.79)	1.16 (0.45 to 3.03)	0.44 (0.05 to 3.50)
Detection mode: Clinical cancer in women without previous mammograms	2.61 (0.81 to 8.37)	0.66 (0.20 to 2.12)	0.35 (0.05 to 2.38)
Detection mode: Clinical cancer in women who had previous mammograms (i.e. interval >24 months)	3.54 (1.15 to 10.89)	1.57 (0.63 to 3.94)	0.34 (0.06 to 2.02)
ER status: Negative	5.19 (2.68 to 10.06)	1.17 (0.48 to 2.87)	0.22 (0.07 to 0.77)
Grade: Intermediate-differentiated	1.97 (0.24 to 16.23)	1.82 (0.52 to 6.34)	1.32 (0.10 to 18.26)
Grade: Poorly-differentiated	7.11 (0.91 to 55.30)	1.55 (0.39 to 6.22)	0.36 (0.03 to 4.92)
Tumor size: ≥ 20	0.87 (0.48 to 1.59)	1.26 (0.67 to 2.39)	1.17 (0.37 to 3.76)
Nodal involvement: Yes	1.60 (0.79 to 3.27)	2.54 (1.20 to 5.37)	1.67 (0.43 to 6.51)
<i>Model 3: Breast cancer subtype, adjusted for age and year of diagnosis</i>			
Subtype: Luminal B	2.83 (0.54 to 14.77)	0.49 (0.06 to 3.73)	0.19 (0.01 to 2.60)
Subtype: HER2-enriched	0.93 (0.11 to 8.07)	0.33 (0.04 to 2.52)	0.38 (0.02 to 8.07)
Subtype: Basal-like	40.07 (14.26 to 112.59)	0.84 (0.11 to 6.43)	0.02 (0.00 to 0.17)

Table 2. Frequency, odds ratio (OR) and corresponding 95% confidence intervals (CI) of patient characteristics among *BRCA* carriers identified versus not identified through selective clinical screening.

* Adjusted for year of diagnosis (2001-2004, 2005-2008). † Adjusted for year of diagnosis and gene (*BRCA1*, *BRCA2*). ‡ Adjust for year of diagnosis, gene and age at diagnosis (<50, 50-59, ≥60).

Patient characteristic	Not identified by selective testing (n=57) n (%)	Identified by selective testing (n=35) n (%)	OR (95% CI)*	OR (95% CI)†	OR (95% CI)‡
Gene, *unadjusted					
<i>BRCA1</i>	23 (40.4)	27 (77.1)	1.00 (Reference)		
<i>BRCA2</i>	34 (59.6)	8 (22.9)	0.20 (0.08 to 0.52)		
Age at diagnosis, *unadjusted					
<50	15 (26.3)	22 (62.9)	1.00 (Reference)		
50-59	20 (35.1)	8 (22.9)	0.27 (0.10 to 0.78)		
≥60	22 (38.6)	5 (14.3)	0.15 (0.05 to 0.50)		
Year of diagnosis, *unadjusted					
2001-2004	26 (45.6)	12 (34.3)	1.00 (Reference)		
2005-2008	31 (54.4)	23 (65.7)	1.61 (0.67 to 3.84)		
Education					
University	29 (50.9)	21 (60.0)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Intermediate	12 (21.1)	9 (25.7)	1.06 (0.37 to 2.98)	1.40 (0.45 to 4.39)	2.08 (0.59 to 7.40)
Elementary	7 (12.3)	0 (0.0)	-	-	-
Other	9 (15.8)	5 (14.3)	0.78 (0.23 to 2.68)	0.65 (0.17 to 2.46)	1.63 (0.35 to 7.66)
Age at menarche in years					
<13	21 (36.8)	7 (20.0)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
≥13	36 (63.2)	28 (80.0)	2.17 (0.79 to 5.94)	2.99 (1.00 to 8.94)	4.12 (1.19 to 14.26)
Menopause status before breast cancer diagnosis					
Premenopause	14 (24.6)	23 (65.7)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Postmenopause	43 (75.4)	12 (34.3)	0.17 (0.07 to 0.44)	0.17 (0.06 to 0.45)	0.18 (0.03 to 1.25)
BMI in kg/m ²					
<25	29 (50.9)	24 (68.6)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
≥25	27 (47.4)	11 (31.4)	0.52 (0.21 to 1.27)	0.42 (0.16 to 1.12)	0.32 (0.11 to 0.94)
Missing	1 (1.8)	0 (0.0)			
Percentage mammographic density					
<25	22 (38.6)	10 (28.6)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
≥25	16 (28.1)	14 (40.0)	1.97 (0.69 to 5.62)	1.54 (0.51 to 4.69)	0.93 (0.27 to 3.21)
Missing	19 (33.3)	11 (31.4)			
Number of children					
0	12 (21.1)	3 (8.6)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
1	13 (22.8)	7 (20.0)	2.39 (0.49 to 11.65)	2.64 (0.50 to 13.83)	5.34 (0.84 to 33.79)
2	22 (38.6)	14 (40.0)	2.91 (0.68 to 12.53)	3.12 (0.68 to 14.24)	4.76 (0.89 to 25.43)
≥3	10 (17.5)	11 (31.4)	4.64 (1.00 to 21.66)	4.69 (0.93 to 23.60)	10.55 (1.62 to 68.68)
HRT ever					
No	34 (59.6)	25 (71.4)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Yes	21 (36.8)	10 (28.6)	0.61 (0.24 to 1.54)	0.45 (0.16 to 1.24)	0.84 (0.26 to 2.70)
Missing	2 (3.5)	0 (0.0)			
Oral contraceptives ever					
No	19 (33.3)	5 (14.3)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Yes	37 (64.9)	30 (85.7)	3.04 (1.01 to 9.15)	2.90 (0.91 to 9.24)	2.36 (0.71 to 7.85)
Missing	1 (1.8)	0 (0.0)			
Family history of breast cancer					
No	37 (64.9)	18 (51.4)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Yes	20 (35.1)	17 (48.6)	1.84 (0.77 to 4.39)	1.58 (0.63 to 3.99)	1.46 (0.54 to 3.90)

Family history of ovarian cancer					
No	50 (87.7)	24 (68.6)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Yes	7 (12.3)	11 (31.4)	3.11 (1.06 to 9.09)	2.87 (0.91 to 9.11)	3.41 (0.99 to 11.73)
Ovarian cancer					
No	51 (89.5)	33 (94.3)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Yes	6 (10.5)	2 (5.7)	0.60 (0.11 to 3.26)	0.37 (0.06 to 2.17)	0.46 (0.07 to 3.01)
Other malignant cancer					
No	48 (84.2)	31 (88.6)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Yes	9 (15.8)	4 (11.4)	0.76 (0.21 to 2.75)	0.54 (0.14 to 2.12)	0.66 (0.15 to 2.96)

**Prevalence of *BRCA1* and *BRCA2* pathogenic variants in a
large, unselected breast cancer cohort**

DATA SUPPLEMENT 1

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eTable 1. Exclusion criteria for genotyping experiment (Michailidou *et al.* 1). Of the 5,715 women who consented to genetic analyses of their blood samples, genotyping was successfully performed for 5,125 women. Of these, 5,122 had enough DNA remaining for targeted sequencing.

Exclusion criteria for genotyping experiment	<i>n</i>
Concordant replicate - exclude lower call rate	116
Cryptic Duplicate	7
Extreme heterozygous	34
Call rate (<95%)	8
Male	1
Non-European	114
Phenotype data excluded	177
Relative pairs with different status	9
Relative pairs, exclude lower call rate	39
Unclear whether consented when data released in Jan 2012	69
Study duplicates with KARBAC sample	4
Genotype not received	12

eMETHODS

Details on targeted sequencing methodology used by the University of Cambridge (Fluidigm Access Array method)

Targeted sequencing

Target-enriched sequencing libraries of germline DNA from 5,122 breast cancer patients were prepared at the Centre for Cancer Genetic Epidemiology (University of Cambridge). Data used in this study were part of a larger effort that included samples from other cohorts, as well as coding sequences and intron/exon boundaries for a total of 31 known or suspected breast cancer susceptibility genes, including *BRCA1* and *BRCA2*. Assay design was conducted as previously described². See eTable 2 (Data Supplement 2) for primer sequences and amplicon details.

Briefly, target sequence enrichment was performed using 48.48 Fluidigm Access Arrays according to the manufacturer's protocol (Fluidigm, South San Francisco, California, USA). Fluidigm D3 assay design software was used to select primer pairs, which were multiplexed into pools selected for GC content and avoidance of off-target primer-primer and primer-product complementarity. Target sequences were amplified with Illumina sequencing adaptors and one of 1,536 unique sample barcodes (supplied by Fluidigm, South San Francisco, California, USA). Robotic liquid handling and barcode plate identification were used in all steps of the library preparation process. Each library of amplicons (eTable 2, Data Supplement 2) for 1,536 samples was quantified with the KAPA Library Quantification Kit (KapaBiosystems, Boston, Massachusetts, USA) and then sequenced on the Illumina Hi-Seq 2500 instrument using v4 chemistry, according to the manufacturer's protocol (Illumina, San Diego, California, USA). Each library was sequenced 2-3 times to provide sufficient coverage.

Sequence data processing and quality control

Raw data in FASTQ format was received from the University of Cambridge. Paired-end sequencing reads were aligned to the human genome reference sequence (hg19) using Burrows-Wheeler Aligner (version 0.7.12³). Aligned reads in SAM format were converted to BAM format and subsequently merged for each sample using SAMtools (version 1.1⁴). Read groups were assigned using Picard (version 1.119; <http://broadinstitute.github.io/picard>). Genome Analysis Toolkit (GATK version 3.7.0; <https://software.broadinstitute.org/gatk/>) was used for local insertion/deletion (indel) realignment and base quality score recalibration, variant calling, SNP and indel parsing and for deriving quality and depth metrics⁵. The mean read depth across the coding sequences of *BRCA1* and *BRCA2* was 792.2 (standard deviation: 587.4) and 631 (standard deviation: 516), respectively. More than 90% of targeted bases had more than 15x coverage (94.8 [15.9] and 92.5 [20.4] for *BRCA1* and *BRCA2*, respectively).

Genetic variants were called with Unified Genotyper using the default parameters except –mindelFrac 0.05. SNPs and indels with low variant confidence/quality by depth (QD<2) and low approximate read depth (DP<10) were removed. Filter-based annotation of variants were performed using ANNOVAR⁶. A total of 5,099 samples with valid variant calls were included in the final analytical dataset.

Details on targeted sequencing methodology used by the Department of Oncology, Lund University (modified SureSelect hybrid selection method)

The clinical mutation screening was performed using the most sensitive methods available for comprehensive detection of all classes of genetic variants known to affect *BRCA1* and *BRCA2*. Targeted sequencing libraries were prepared using a modified SureSelect hybrid selection method and a custom panel targeting 64 genes including complete *BRCA1* and *BRCA2* loci (exons and introns) and 100kb up- and downstream. Specificity was ensured by confirming all variants with Sanger sequencing on an independent DNA extraction from the patient blood sample. Paired-end sequencing of the libraries was performed on a HiSeq 2500 (2x100bp) to an average depth of ~400 reads. Until 2016, this was complemented with multiple ligation-dependent probe amplification (MLPA) for detection of deletions and duplications affecting one or more complete exons. The lab now has validated bioinformatic methods for detecting these variants directly from the sequencing data. Sensitivity estimated using a large collection of positive control samples including all classes of known pathogenic variants is 100%. Before 2010, denaturing high performance liquid chromatography (DHPLC) and MLPA was used. Together, the DHPLC and MLPA have a stated sensitivity of 95%. Many of the samples tested before 2010 have been screened again using the latest methods.

eTable 3. Swedish Breast Cancer Group criteria for recommending *BRCA1/2* testing.

Criterion	Number meeting criterion
Three cases of breast cancer in first degree relatives, or second degree relatives thought a male, with at least one diagnosed ≤50y, and/or ovarian cancer (regardless of age)	79
Two cases of breast cancer or ovarian cancer in first degree relatives, or second degree relatives thought a male, with at least one case of breast cancer diagnosed ≤40y, or two cases of ovarian cancer (regardless of age)	113
One case of breast cancer ≤35y	99
One case of triple-negative breast cancer ≤40y	20
One case of male breast cancer	NA
Breast cancer and ovarian cancer in one individual	44
Cases of bilateral breast cancer, prostate cancer, and pancreatic cancer may strengthen the indication for screening of pathogenic variants in <i>BRCA1</i> and <i>BRCA2</i> , but are not defined in any specific criterion	NA
Total	298

eTable 4. Description of *BRCA1* (NM_007294.3) pathogenic variants.

Exon	cDNA Change	AA Change	Variant Classification	BIC Nomenclature	Note	n
2	c.68_69delAG	p.E23fs	frameshift deletion	185_186delAG,185delAG,187delAG	Founder mutation in Ashkenazi Jews ⁷	3
5	c.181T>G	p.C61G	nonsynonymous SNV	300T>G	Common mutation in Europe ⁸	1
7	c.302-2A>G	-	splice site	-	-	1
11	c.930delG	p.Q310fs	frameshift deletion	1049delG	-	1
11	c.962G>A	p.W321*	stopgain	W321X	-	1
11	c.1082_1092delCAGAGAACCT	p.S361*	stopgain	1201del11	Founder mutation common in Southern Sweden ⁹	5
11	c.1360_1361delAG	p.S454*	stopgain	1479delAG	-	3
11	c.1504_1508delTTAAA	p.L502fs	frameshift deletion	1623_1627delTTAAA	-	1
11	c.1772delT	p.I591fs	frameshift deletion	1891delT	-	1
11	c.1961delA	p.K654fs	frameshift deletion	2080delA	-	1
11	c.2184delA	p.E729fs	frameshift deletion	-	-	1
11	c.2475delC	p.D825fs	frameshift deletion	2594delC	Swedish BRCA1 founder mutation ¹⁰	2
11	c.3048_3052dupTGAGA	p.N1018fs	frameshift insertion	3166insTGAGA, p.Asn1018fs	Founder mutation originating from West Coast of Sweden ^{8, 11}	8
11	c.3178G>T	p.E1060*	stopgain	E1060X	-	1
11	c.3485delA	p.D1162fs	frameshift deletion	3604delA	Founder mutation in Finland ⁸	1
11	c.3607C>T	p.R1203*	stopgain	3726C>T	-	1
11	c.3626delT	p.L1209*	stopgain	3745delT	Founder mutation originating in Northern Sweden ⁸	3
11	c.3700_3704delGTAAA	p.V1234fs	frameshift deletion	3819_3823delGTAAA	Frequent mainly in Middle and Eastern Europe and Canada ¹²	1
11	c.4035delA	p.E1346fs	frameshift deletion	4154delA	Common mutation in Poland and Latvia ⁸	2
13	c.4201C>T	p.Q1401*	stopgain	-	-	1
13	c.4327C>T	p.R1443*	stopgain	4446C>T	-	1
17	c.5030_5033delCTAA	p.T1677fs	frameshift deletion	5149del4,5147del4,5146del4	-	1
18	c.5075-2A>C	-	splice site	IVS17-2A>C	-	1
18	c.5095C>T	p.R1699W	nonsynonymous SNV	5214C>T	-	1
18	c.5123C>A	p.A1708E	nonsynonymous SNV	5242C>A	-	1
19	c.5153-1G>C	-	splice site	IVS18-1G>C	-	2
20	c.5266dupC	p.Q1756fs	frameshift insertion	5382_5383insC,5382insC,5383insC,5384insC,5385insC	Founder mutation in Russia ¹³	3
21	c.5278-2A>T	-	splice site	-	-	1

eTable 5. Description of *BRCA2* (NM_000059.3) pathogenic variants.

Exon	cDNA Change	AA Change	Variant Classification	BIC Nomenclature	Note	n
10	c.805dupA	p.T269fs	frameshift insertion	1033insA,p.Thr269fs	-	1
10	c.1310_1313delAAGA	p.K437fs	frameshift deletion	1537_1540delAAAG	-	1
10	c.1796_1800delCTTAT	p.S599*	stopgain	2024_2028delCTTAT	-	1
10	c.1813dupA	p.I605fs	frameshift insertion	2041_2042insA	-	1
11	c.2179delT	p.S727fs	frameshift deletion	-	-	1
11	c.2376C>G	p.Y792*	stopgain	-	-	1
11	c.2476G>T	p.E826*	stopgain	-	-	1
11	c.2578delA	p.I860fs	frameshift deletion	-	-	1
11	c.2808_2811delACAA	p.A938fs	frameshift deletion	3036_3039delACAA	-	1
11	c.3157_3163delTTAGATA	p.L1053fs	frameshift deletion	-	-	1
11	c.3283C>T	p.Q1095*	stopgain	-	-	2
11	c.3847_3848delGT	p.V1283fs	frameshift deletion	4075_4076delGT	-	1
11	c.3860delA	p.N1287fs	frameshift deletion	4088delA,4082delA	-	1
11	c.3950delC	p.T1317fs	frameshift deletion	-	-	1
11	c.5073delA	p.K1691fs	frameshift deletion	5301delA	-	3
11	c.5754_5755delTA	p.H1918fs	frameshift deletion	-	-	2
11	c.5823delA	p.V1942fs	frameshift deletion	6051delA	-	1
11	c.5946delT	p.S1982fs	frameshift deletion	6174delT	Founder mutation in Ashkenazi Jews ⁸	4
11	c.6444delT	p.I2149fs	frameshift deletion	-	-	1
11	c.6486_6489delACAA	p.K2162fs	frameshift deletion	6714_6717delACAA	-	2
14	c.7097dupT	p.T2367fs	frameshift insertion	-	-	1
14	c.7414_7415delAA	p.K2472fs	frameshift deletion	7642delAA	-	1
15	c.7443delT	p.T2482fs	frameshift deletion	7671delT	-	1
15	c.7480C>T	p.R2494*	stopgain	7708C>T	-	1
15	c.7558C>T	p.R2520*	stopgain	7786C>T	-	1
16	c.7618-1G>A	-	splice site	IVS15-1G>A	-	1
17	c.7974C>G	p.Y2658*	stopgain	Y2658X	-	1
19	c.8332-1G>A	-	splice site	-	-	1
20	c.8513T>G	p.L2838*	stopgain	8741T>G, p.Leu2838X	-	1
22	c.8910G>A	p.W2970*	stopgain	9138G>A (W-X),p.Trp2970X	-	1
23	c.9097delA	p.T3033fs	frameshift deletion	-	-	2
24	c.9118-2A>G	-	splice site	IVS23-2A>G	-	1
25	c.9403delC	p.L3135fs	frameshift deletion	9631delC	-	1

eTable 6. Frequency, odds ratio (OR) and corresponding 95% confidence intervals (CI) of patient characteristics according to *BRCA* status. *Adjusted for age (<50, 50-59, ≥60) and year of diagnosis (2001-2004 and 2005-2008).

Patient characteristic	Non- <i>BRCA</i> (n=5,007)	<i>BRCA1</i> (n=50)	<i>BRCA2</i> (n=42)	<i>BRCA1 vs non-BRCA</i> OR (95% CI)*	<i>BRCA2 vs</i> <i>non-BRCA</i> OR (95% CI)*	<i>BRCA2 vs BRCA1</i> OR (95% CI)*
Age at study entry, years (mean, SD)	63.4 (9.9)	54.9 (12.6)	58.6 (9.4)			
Age at diagnosis, years (mean, SD)	58.6 (9.9)	50.3 (12.4)	54.0 (9.5)			
Age at diagnosis, years (unadjusted)						
<50	887 (17.7)	24 (48.0)	13 (31.0)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
50-59	1666 (33.3)	13 (26.0)	15 (35.7)	0.29 (0.15 to 0.57)	0.61 (0.29 to 1.30)	2.13 (0.78 to 5.81)
≥60	2454 (49.0)	13 (26.0)	14 (33.3)	0.20 (0.10 to 0.39)	0.39 (0.18 to 0.83)	1.99 (0.72 to 5.47)
Year of diagnosis (unadjusted)						
2001-2004	2325 (46.4)	19 (38.0)	19 (45.2)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
2005-2008	2682 (53.6)	31 (62.0)	23 (54.8)	1.41 (0.80 to 2.51)	1.05 (0.57 to 1.93)	0.74 (0.32 to 1.71)
Education						
University	2113 (42.2)	29 (58.0)	21 (50.0)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Intermediate	1116 (22.3)	9 (18.0)	12 (28.6)	0.60 (0.28 to 1.27)	1.09 (0.53 to 2.23)	1.51 (0.52 to 4.42)
Elementary	753 (15.0)	3 (6.0)	4 (9.5)	0.48 (0.14 to 1.63)	0.66 (0.22 to 1.96)	1.10 (0.20 to 6.02)
Other	961 (19.2)	9 (18.0)	5 (11.9)	1.05 (0.48 to 2.29)	0.63 (0.23 to 1.72)	0.50 (0.13 to 1.89)
Missing	64 (12.8)	0 (0.0)	0 (0.0)			
Age at menarche, years						
<13	1592 (31.8)	17 (34.0)	11 (26.2)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
≥13	3263 (65.2)	33 (66.0)	31 (73.8)	1.12 (0.62 to 2.03)	1.52 (0.76 to 3.06)	1.51 (0.58 to 3.89)
Missing	152 (3.0)	0 (0.0)	0 (0.0)			
BMI, kg/m ²						
<25	2644 (52.8)	28 (56.0)	25 (59.5)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
≥25	2275 (45.4)	22 (44.0)	16 (38.1)	1.03 (0.58 to 1.81)	0.79 (0.42 to 1.49)	0.84 (0.35 to 2.00)
Missing	88 (1.8)	0 (0.0)	1 (2.4)			
Percentage mammographic density						
<25	2362 (47.2)	15 (30.0)	17 (40.5)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
≥25	1507 (30.1)	20 (40.0)	10 (23.8)	1.34 (0.66 to 2.75)	0.73 (0.32 to 1.65)	0.52 (0.18 to 1.53)
Missing	1138 (22.7)	15 (30.0)	15 (35.7)			
Number of children						
0	814 (16.3)	8 (16.0)	7 (16.7)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
1	887 (17.7)	10 (20.0)	10 (23.8)	1.26 (0.49 to 3.21)	1.37 (0.52 to 3.62)	0.96 (0.24 to 3.85)
2	2145 (42.8)	19 (38.0)	17 (40.5)	0.98 (0.43 to 2.26)	0.96 (0.40 to 2.33)	0.95 (0.27 to 3.31)
≥3	1130 (22.6)	13 (26.0)	8 (19.0)	1.36 (0.56 to 3.31)	0.89 (0.32 to 2.48)	0.64 (0.16 to 2.54)
Missing	31 (0.6)	0 (0.0)	0 (0.0)			
HRT ever						
No	2208 (44.1)	30 (60.0)	29 (69.0)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Yes	2694 (53.8)	20 (40.0)	11 (26.2)	1.02 (0.53 to 1.94)	0.36 (0.17 to 0.75)	0.36 (0.13 to 1.00)
Missing	105 (2.1)	0 (0.0)	2 (4.8)			
Oral contraceptives ever						
No	1285 (25.7)	11 (22.0)	13 (31.0)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Yes	3663 (73.2)	39 (78.0)	28 (66.7)	0.87 (0.43 to 1.75)	0.58 (0.29 to 1.16)	0.67 (0.26 to 1.75)
Missing	59 (1.1)	0 (0.0)	1 (2.4)			
Ovarian cancer						
No	4971 (99.3)	44 (88.0)	40 (95.2)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Yes	36 (0.7)	6 (12.0)	2 (4.8)	28.02 (10.72 to 73.29)	8.11 (1.87 to 35.24)	0.27 (0.05 to 1.50)
Any malignant cancer, not breast						
No	4494 (89.8)	41 (82.0)	38 (90.5)			
Yes	513 (10.2)	9 (18.0)	4 (9.5)	2.93 (1.37 to 6.27)	1.12 (0.39 to 3.20)	0.39 (0.10 to 1.44)

Family history of breast cancer						
No	3948 (78.8)	27 (54.0)	28 (66.7)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Yes	916 (18.3)	23 (46.0)	14 (33.3)	4.00 (2.27 to 7.05)	2.23 (1.17 to 4.26)	0.60 (0.25 to 1.43)
Missing	143 (2.9)	0 (0.0)	0 (0.0)			
Family history of ovarian cancer						
No	4753 (94.9)	38 (76.0)	36 (85.7)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Yes	231 (4.6)	12 (24.0)	6 (14.3)	7.53 (3.82 to 14.82)	3.62 (1.50 to 8.71)	0.52 (0.17 to 1.61)
Missing	23 (0.5)	0 (0.0)	0 (0.0)			
Breast cancer in mother						
No	4392 (87.7)	29 (58.0)	32 (76.2)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Yes	579 (11.6)	21 (42.0)	10 (23.8)	5.17 (2.92 to 9.17)	2.29 (1.12 to 4.68)	0.47 (0.18 to 1.20)
Missing	36 (0.7)	0 (0.0)	0 (0.0)			
Age at breast cancer diagnosis in mother						
<50	92 (15.9)	11 (52.4)	4 (40.0)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
≥59	446 (77.0)	9 (42.9)	6 (60.0)	0.20 (0.08 to 0.50)	0.37 (0.10 to 1.35)	2.05 (0.39 to 10.67)
Missing	43 (7.4)	1 (4.8)	0 (0.0)			
Ovarian cancer in mother						
No	4822 (96.3)	39 (78.0)	36 (85.6)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Yes	149 (3.0)	11 (22.0)	6 (14.3)	9.82 (4.85 to 19.89)	5.44 (2.24 to 13.18)	0.61 (0.20 to 1.86)
Missing	36 (0.7)	0 (0.0)	0 (0.0)			
Ovarian cancer in sister						
No	4885 (97.6)	48 (96.0)	42 (100.0)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Yes	86 (1.7)	2 (4.0)	0 (0.0)	3.23 (0.76 to 13.76)	-	-
Missing	36 (0.7)	0 (0.0)	0 (0.0)			

eTable 7. Frequency, odds ratio (OR) and corresponding 95% confidence intervals (CI) of tumor characteristics according to *BRCA* status. *Adjusted for age (<50, 50-59, ≥60) and year of diagnosis (2001-2004 and 2005-2008).

Tumor characteristic	Non- <i>BRCA</i> (n=5,007) n (%)	<i>BRCA1</i> (n=50) n (%)	<i>BRCA2</i> (n=42) n (%)	<i>BRCA1</i> vs <i>non-BRCA</i> OR (95% CI)*	<i>BRCA2</i> vs <i>non-BRCA</i> OR (95% CI)*	<i>BRCA2</i> vs <i>BRCA1</i> OR (95% CI)*
Type of breast cancer						
Invasive	4470 (89.3)	48 (96.0)	42 (100.0)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Non-invasive	522 (10.4)	2 (4.0)	0 (0.0)	0.37 (0.09 to 1.53)	-	-
Missing	15 (0.3)	0 (0.0)	0 (0.0)			
Detection mode						
Screen-detected	1844 (36.8)	5 (10.0)	12 (28.6)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Interval	768 (15.3)	5 (10.0)	7 (16.7)	2.36 (0.68 to 8.17)	1.39 (0.54 to 3.54)	0.63 (0.12 to 3.20)
Clinical cancer in women without previous mammograms	911 (18.2)	8 (16.0)	4 (9.5)	3.99 (1.26 to 12.66)	0.76 (0.24 to 2.43)	0.22 (0.04 to 1.08)
Clinical cancer in women who had previous mammograms (i.e. interval >24 months)	1395 (27.9)	31 (62.0)	19 (45.2)	5.20 (1.78 to 15.15)	1.77 (0.73 to 4.29)	0.35 (0.08 to 1.49)
Missing	89 (1.8)	1 (2.0)	0 (0.0)			
Estrogen receptor status						
Positive	3637 (72.6)	17 (34.0)	30 (71.4)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Negative	643 (12.8)	30 (60.0)	7 (16.7)	8.98 (4.90 to 16.46)	1.23 (0.54 to 2.82)	0.14 (0.05 to 0.39)
Missing	727 (14.5)	3 (6.0)	5 (11.9)			
Progesterone receptor status						
Positive	2952 (59.0)	14 (28.0)	24 (57.1)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Negative	1252 (25.0)	33 (66.0)	13 (31.0)	6.06 (3.21 to 11.46)	1.33 (0.67 to 2.63)	0.23 (0.09 to 0.60)
Missing	803 (16.0)	3 (6.0)	5 (11.9)			
Grade						
Well-differentiated	578 (11.5)	1 (2.0)	3 (7.1)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Moderately differentiated	1563 (31.2)	7 (14.0)	16 (38.1)	2.41 (0.30 to 19.66)	1.91 (0.55 to 6.60)	0.80 (0.07 to 9.47)
Poorly differentiated	822 (16.4)	31 (62.0)	9 (21.4)	17.99 (2.44 to 132.70)	1.90 (0.51 to 7.10)	0.11 (0.01 to 1.22)
Missing	2044 (40.8)	11 (22.0)	14 (33.3)			
Tumor size (mm)						
<20	3020 (60.3)	27 (54.0)	23 (54.8)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
≥20	1608 (32.1)	21 (42.0)	19 (45.2)	1.30 (0.73 to 2.32)	1.47 (0.80 to 2.72)	1.16 (0.46 to 2.89)
Missing	379 (7.6)	2 (4.0)	0 (0)			
Nodal involvement						
No	4503 (89.9)	39 (78.0)	32 (76.2)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Yes	466 (9.3)	11 (22.0)	10 (23.8)	2.08 (1.04 to 4.14)	2.71 (1.31 to 5.62)	1.27 (0.46 to 3.54)
Missing	38 (0.8)	0 (0.0)	0 (0.0)			
Proliferation level (Ki67)						
Low (<20%)	923 (18.4)	5 (10.0)	11 (26.2)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
High (≥20%)	736 (14.7)	20 (40.0)	7 (16.7)	4.25 (1.58 to 11.44)	0.72 (0.28 to 1.88)	0.18 (0.04 to 0.74)
Missing	3348 (66.9)	25 (50.0)	24 (57.1)			
Molecular subtypes						
Luminal A	1212 (24.2)	5 (10.0)	15 (35.7)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Luminal B	156 (3.1)	2 (4.0)	1 (2.4)	2.83 (0.54 to 14.77)	0.49 (0.06 to 3.73)	0.19 (0.01 to 2.60)
HER2-enriched	214 (4.3)	1 (2.0)	1 (2.4)	0.93 (0.11 to 8.07)	0.33 (0.04 to 2.52)	0.38 (0.02 to 8.07)
Basal-like	84 (1.7)	17 (34.0)	1 (2.4)	40.07 (14.26 to 112.59)	0.84 (0.11 to 6.43)	0.02 (0.00 to 0.17)
Missing	3341 (66.7)	25 (50.0)	24 (57.1)			

eTable 8. Frequency, odds ratio (OR) and corresponding 95% confidence intervals (CI) of tumor characteristics among *BRCA* carriers identified versus not identified through selective clinical screening. * Adjusted for year of diagnosis (2001-2004, 2005-2008). † Adjusted for year of diagnosis and gene (*BRCA1*, *BRCA2*). ‡ Adjust for year of diagnosis, gene and age at diagnosis (<50, 50-59, ≥60).

Tumor characteristic	Not identified by selective testing (n=57) n (%)	Identified by selective testing (n=35) n (%)	OR (95% CI)*	OR (95% CI)†	OR (95% CI)‡
Type of breast cancer					
Invasive	56 (98.2)	34 (97.1)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Non-invasive	1 (1.8)	1 (2.9)	2.27 (0.13 to 39.73)	1.11 (0.06 to 20.11)	1.44 (0.06 to 37.74)
Detection mode					
Screen-detected	14 (24.6)	3 (8.6)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Interval	8 (14.0)	4 (11.4)	2.56 (0.44 to 14.85)	2.24 (0.34 to 14.73)	1.56 (0.21 to 11.33)
Clinical cancer in women without previous mammograms	10 (17.5)	2 (5.7)	0.79 (0.11 to 5.72)	0.41 (0.05 to 3.37)	0.48 (0.06 to 4.06)
Clinical cancer in women who had previous mammograms	24 (42.1)	26 (74.3)	5.52 (1.38 to 22.18)	3.85 (0.88 to 16.87)	1.88 (0.32 to 11.01)
Missing	1 (1.8)	0 (0.0)			
Estrogen receptor					
Positive	34 (59.6)	13 (37.1)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Negative	19 (33.3)	18 (51.4)	2.48 (0.99 to 6.19)	1.29 (0.45 to 3.68)	0.81 (0.25 to 2.63)
Missing	4 (7.0)	4 (11.4)			
Progesterone receptor					
Positive	25 (43.9)	13 (37.1)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Negative	27 (47.4)	19 (54.3)	1.30 (0.53 to 3.19)	0.69 (0.24 to 1.97)	0.46 (0.14 to 1.52)
Missing	5 (8.8)	3 (8.6)			
Grade					
Poorly-differentiated	20 (35.1)	20 (57.1)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Intermediate-differentiated	18 (31.6)	5 (14.3)	0.28 (0.08 to 0.92)	0.48 (0.13 to 1.78)	0.67 (0.17 to 2.70)
Well-differentiated	4 (7.0)	0 (0.0)	-	-	-
Missing	15 (26.3)	10 (28.6)			
Tumor size (mm)					
<20	35 (61.4)	15 (42.9)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
≥20	16 (28.1)	17 (48.6)	2.48 (1.00 to 6.16)	2.91 (1.07 to 7.92)	2.15 (0.74 to 6.24)
Missing	5 (8.8)	2 (5.7)			
Nodal involvement					
No	45 (78.9)	26 (74.3)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Yes	12 (21.1)	9 (25.7)	1.40 (0.51 to 3.84)	1.53 (0.52 to 4.52)	1.15 (0.36 to 3.67)
Proliferation level (Ki67)					
Low (<20%)	11 (19.3)	5 (14.3)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
High (≥20%)	12 (21.1)	15 (42.9)	2.75 (0.75 to 10.11)	1.55 (0.37 to 6.43)	0.80 (0.16 to 3.96)
Missing	34 (59.6)	15 (42.9)			
Molecular subtypes					
Luminal A	14 (24.6)	6 (17.1)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Luminal B	2 (3.5)	1 (2.9)	1.17 (0.09 to 15.46)	0.65 (0.04 to 9.93)	0.37 (0.02 to 6.64)
HER2-enriched	2 (3.5)	0 (0.0)	-	-	-
Basal-like	5 (8.8)	13 (37.1)	6.07 (1.49 to 24.76)	2.54 (0.52 to 12.41)	1.49 (0.25 to 8.76)
Missing	34 (59.6)	15 (42.9)			

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eTable 2. Primer sequences and amplicon details. Partially overlapping amplicons were tiled across the target regions with a maximum amplicon length (forward and reverse primer length plus intervening unique sequence) of 200-bp. Primers were designed with melting temperature range 59.0-61.0 °C. Each primer included an orientation-specific tail sequence for subsequent ligation of adapter and barcode sequences.

BRCA1	BRCA1_Intron_13_3	17	41231596	41231778	ACACTGACGACATGGTCTA CAGCACATTAAACACTCTAA GAGCCAA	TACGGTAGCAGAGACTTGGTCTG GAATAGGTTCCAGCTGCTCA	183	48
BRCA1	BRCA1_Intron_13_4	17	41231671	41231869	ACACTGACGACATGGTCTA CAAAAGAGAAAGGTACGTG GGTC	TACGGTAGCAGAGACTTGGTCTT GTTCTGGTGTAGAATGACTTTTC	199	41
BRCA1	BRCA1_Intron_13_5	17	41231760	41231936	ACACTGACGACATGGTCTA CAAGCAGCTGGAACCTATTCC CCTAT	TACGGTAGCAGAGACTTGGTCTA CAAGTTATCTTATTGCTTATTG CAT	177	32
BRCA1	BRCA1_Intron_13_6	17	41231851	41232048	ACACTGACGACATGGTCTA CATCATTCTATACCGAAC ATTAGCA	TACGGTAGCAGAGACTTGGTCTC ACAAGGCTTAGGTTGGCCTT	198	35
BRCA1	BRCA1_Intron_13_7	17	41231905	41232081	ACACTGACGACATGGTCTA CACCTCATGCAATGAAGCAA ATAAGAT	TACGGTAGCAGAGACTTGGTCTT TCCTAACACAGCCAGCAAGTAG	177	37
BRCA1	BRCA1_Intron_13_8	17	41232004	41232201	ACACTGACGACATGGTCTA CATCCTTAATTCTATTCTGA ACAGCA	TACGGTAGCAGAGACTTGGTCTT GCCGCATACTTCTATCCACAA	198	39
BRCA1	BRCA1_17	17	41234313	41234511	ACACTGACGACATGGTCTA CAGACAAGGATCATAAAAT GTTGAG	TACGGTAGCAGAGACTTGGTCTA GCCAGCCTCTAACAGCTAC	199	43
BRCA1	BRCA1_18	17	41234442	41234625	ACACTGACGACATGGTCTA CACTGGATTCGCAAGTCCT CAAG	TACGGTAGCAGAGACTTGGTCTT CATTCTGGTGCCTATTATCGT	184	46
BRCA1	BRCA1_Intron_12_regi on_2_1	17	41236576	41236770	ACACTGACGACATGGTCTA CAACTTCCGTTCTCTTCT GATCC	TACGGTAGCAGAGACTTGGTCTA ATGGCATTGTCAGACCTT	195	40
BRCA1	BRCA1_Intron_12_regi on_2_2	17	41236708	41236884	ACACTGACGACATGGTCTA CATCACAAAGGTTCTACTG CTACTCT	TACGGTAGCAGAGACTTGGTCTC CTGTGAAGCTTTGGTCAGAT	177	46
BRCA1	BRCA1_Intron_12_regi on_2_3	17	41236803	41236999	ACACTGACGACATGGTCTA CAACTGATCAAACCTAGCC AAAGT	TACGGTAGCAGAGACTTGGTCTT TGGAACCCAAAGGAAGTTA	197	40
BRCA1	BRCA1_Intron_12_regi on_1_1	17	41237464	41237643	ACACTGACGACATGGTCTA CATGCCATTAGGATAAAAATT CCTCAACA	TACGGTAGCAGAGACTTGGTCTA AAAGAGGAGTGGCAGCAAGTAA	180	41
BRCA1	BRCA1_Intron_12_regi on_1_2	17	41237543	41237725	ACACTGACGACATGGTCTA CACCCCTCACACAAGTTGTT TTCT	TACGGTAGCAGAGACTTGGTCTT CATATTCACAACCTCCCTGTT	183	42
BRCA1	BRCA1_Intron_12_regi on_1_3	17	41237628	41237825	ACACTGACGACATGGTCTA CACTGCCACTCCTTTTC CACT	TACGGTAGCAGAGACTTGGTCTT ATACACTCTGCTCCCTCAGA	198	46
BRCA1	BRCA1_Intron_12_regi on_1_4	17	41237700	41237896	ACACTGACGACATGGTCTA CACCCAGGAGGAGTTGT GAAATA	TACGGTAGCAGAGACTTGGTCTA TACACATGTTATGGGAAGGTT T	197	41
BRCA1	BRCA1_19	17	41242903	41243101	ACACTGACGACATGGTCTA CACTACTGAATGCAAAGGAC ACCAC	TACGGTAGCAGAGACTTGGTCTG CAGCGTTATAGTCTGCTTTAC	199	45
BRCA1	BRCA1_20	17	41243373	41243569	ACACTGACGACATGGTCTA CAATGTGCTCCCCAAAGCA TAAAC	TACGGTAGCAGAGACTTGGTCTC AGTCTGAAAGCCAGGGAGTTG	197	41

Assays designed by
relax mode and have no
off-target hits

BRCA1_3_UTR_Combined	BRCA1_3UTR_Combined_14		17	41196714	41196888	ACACTGACGACATGGTCTCATCTTGAAACCGGTTCTGTG	TACGGTAGCAGAGACTGGTCTAGATCATACCACGGCACTCC	175	35	TCTTGGAAACCGGTTCTGAAAATCTCTGCTGGTTAGAACACATTCTTAGAAATCTGCAAATATCTCAGACTTAACTCTCTAGTTCTA
BRCA1_3_UTR_Combined	BRCA1_3UTR_Combined_15		17	41197039	41197205	ACACTGACGACATGGTCTCATCGAACTCCTGACCTCCAGT	TACGGTAGCAGAGACTGGTCTGTCCTGGCAGTTCTCAA	167	51	TTTCTCTTTTTTTTTTTGAGCCCAGCTTGGCTCCAAAGGGCTCGGAGCTCGCGTGGTAGTGTACT
BRCA1_Intron_2_region_1	BRCA1_Intron_2_region_1_11		17	41271259	41271409	ACACTGACGACATGGTCTCACAGCCTGGTGACAGAGAAT	TACGGTAGCAGAGACTGGTCTTTCACTGCCCTGTGCTATG	151	38	TCGAACCTCTGACCTCAGTGTCTGCCACCTGGCTCCAAAGTGCTGGGATTACAGGCCACATGCCCAAGGAC
BRCA1_Intron_2_region_2	BRCA1_Intron_2_region_2_7		17	41275415	41275564	ACACTGACGACATGGTCTCATGGAGTTCACTGGTGCCTA	TACGGTAGCAGAGACTGGTCTTGAACCCCTCTCCACTAA	150	50	CAGCCTGGTGACAGAGAACATCTCAAAAAAGAAAAAAAGAAAGGATCACAAAGAAAGCTGGGAGACTAACCTTGTGAGGTTGTAACAGGTTGAAATAACAATCTGTGATCATGGGTTTTGACATAGCAGGGCAGTGAAGAAA
BRCA1_Intron_2_region_2	BRCA1_Intron_2_region_2_8		17	41275545	41275700	ACACTGACGACATGGTCTCATAGTGAGAGGGGTTTCA	TACGGTAGCAGAGACTGGTCTCCTACATTGAAATGCCAA	156	53	TGAGGTTAGCTGGTGCATATTGGCTCACAGCAACACTCTGCCCTCTGGTCAAGTGTCTGGGATTACAGCATGCCACTACGCCAGCTAA
BRCA2	BRCA2_Pro_moter_1		13	32888507	32888705	ACACTGACGACATGGTCTCACACCTCTGGAACGAGCA	TACGGTAGCAGAGACTGGTCTCATCTTGATCTTAGGATTGG	199	47	TTAGTGAGAGGGGGTTTACCATGGTGGCCAGGATGGTCTCGATCTCC
BRCA2	BRCA2_Pro_moter_3		13	32888996	32889147	ACACTGACGACATGGTCTCAaaaagcaaaGATACTACCAAGCC	TACGGTAGCAGAGACTGGTCTTGTAGTCCAGTAGCTGTTG	152	46	TGACCTCTGGATCTACCCACCTGGCTCCAAAGTGTGGGATTACAGGCATAAGGCCACCCGGCTCGGCCATCCATGATTTTATTTGCCATTCAAGTGATGG
BRCA2	BRCA2_Pro_moter_6		13	32889044	32889230	ACACTGACGACATGGTCTCATCATGAGCGAGTTAACAGATGGGT	TACGGTAGCAGAGACTGGTCTAAAGCTTAGAGTGGTCCGGTGTG	187	46	CCCCCTGGGAAGCAGGCCCCATGGGAGCAACTCTACTGAATC
BRCA2	BRCA2_prom_Stacey_1		13	32889175	32889357	ACACTGACGACATGGTCTCACCAACTACCAAGTGAAGTCA	TACGGTAGCAGAGACTGGTCTTTGCTCCAGCTCATGTTGG	183	50	CATTGGAGGTTTGTAGGTCTTACAACAAACCTTTCAGCCTGTGTTAAA
BRCA2	BRCA2_prom_Stacey_2		13	32889210	32889390	ACACTGACGACATGGTCTCACACCGACCACTCTAACGCTTTGT	TACGGTAGCAGAGACTGGTCTCCTATGCCACTCCAAGTCCC	181	52	TCATGAGGTTAACAGTGGGTTTCAACATTTCAGCAAGAACAGG
BRCA2	BRCA2_Pro_moter_Combined_7		13	32889335	32889530	ACACTGACGACATGGTCTCACAAACATGAGCTGGAGCAAAA	TACGGTAGCAGAGACTGGTCTATTCTCAGTGGCGAAAGGAA	196	54	GCTCGGAGGTTCTGAACACCTGCTACCCAAATAGCAGAACAGCTACTGGA
BRCA2	BRCA2_Pro_moter_10		13	32889458	32889650	ACACTGACGACATGGTCTCATCCCGCTTATTGGTCAAGTAC	TACGGTAGCAGAGACTGGTCTGCTCTGCCCTAGTT	193	62	ACTAAATCTCTGATTTCAAAATACAGCCCGCCACTACCAACTAAGTG
BRCA2	BRCA2_Pro_moter_Combined_10		13	32889508	32889703	ACACTGACGACATGGTCTCATTCCTTCGCCACACTGAAAT	TACGGTAGCAGAGACTGGTCTCGCAAAGACACCCGAGG	196	67	AAGTCATCCAAACACCCAGGCCCCACTCTAAAGCTTTCCCACCTAAGCTT
BRCA2	BRCA2_5_UTR_exon_1_2		13	32889663	32889835	ACACTGACGACATGGTCTCACGACTGTGCGCCCT	TACGGTAGCAGAGACTGGTCTCTGTCCTAACCCACTACCA	173	62	CCCACACTAAGTGAAGTCATCCAAACACACCGGACCACTTA
BRCA2	BRCA2_prom_Stacey_7		13	32889723	32889921	ACACTGACGACATGGTCTCAGAACGCTGAGGGGACAGATT	TACGGTAGCAGAGACTGGTCTAAGACAAAAGGCAAGAACCC	199	63	AGTGGCTTAACTGGGAGGAGGGGGCTTCCGGCTGGACTCTAACGCTT
BRCA2	BRCA2_prom_Stacey_8		13	32889839	32890018	ACACTGACGACATGGTCTCACGCTTCCGCACTCCAGTC	TACGGTAGCAGAGACTGGTCTGAAATGGAGACCCAGGAAG	180	64	TTCCCTGGCCACACTGAGAAATACCCGAGCGGCCACCCAGGCC
BRCA2	BRCA2_1		13	32890522	32890720	ACACTGACGACATGGTCTCATCCCTGTGAACTGGCTTATGGT	TACGGTAGCAGAGACTGGTCTTTAGAAAACACTTCTCGGTGTA	199	34	ACTTCCGAGGGCCCTTCCCTGGCTCTGGCTACGGCGTACGTGG
BRCA2	BRCA2_2		13	32893174	32893358	ACACTGACGACATGGTCTCATCATCTGGTAAACTAAAGGTGGGA	TACGGTAGCAGAGACTGGTCTAAGATGGTTCTGGAGT	185	32	CCAGCCTGGCTGCGACTCTGCCCTGCGCCTCGGGGTCTT

								TCTGAAACCTGCAGAAGAACATCTGAACATAAAAACAATTACGAACCAA CCTTATTAAAACCACCAAAAGGAAACCATCTTA	
BRCA2	BRCA2_3	13	32893249	32893426	ACACTGACGACATGGTTCTA CACTTCTTCAGAAGCTCCA CCCTA	TACGGTAGCAGAGACTTGGTCTG AGATTGGTACAGCGGCAGAG	178	39	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_4	13	32893336	32893492	ACACTGACGACATGGTTCTA CAACTCCACAAAGGAAACCA TCTTA	TACGGTAGCAGAGACTTGGTCTC TCCCCAGTCTACCATATTGCAT	157	39	One primer sits in the repeat region
BRCA2	BRCA2_Intron_3_1	13	32897972	32898163	ACACTGACGACATGGTTCTA CAACTTAATGCCCTGGAGAG TCAAA	TACGGTAGCAGAGACTTGGTCTA GACAAAGTGAGTTGCGACAGTA	192	29	
BRCA2	BRCA2_Intron_3_2	13	32898044	32898221	ACACTGACGACATGGTTCTA CAGGATTCTATTTGTAAAG CCACCTAT	TACGGTAGCAGAGACTTGGTCTT GTCTGTATTTAGTCACTCCTG AA	178	30	One primer sits in the repeat region
BRCA2	BRCA2_Intron_3_3	13	32898141	32898311	TGCT ACACTGACGACATGGTTCTA CATCCAGTCAATTTTCAGG	TACGGTAGCAGAGACTTGGTCTA CACTGTTCTTTCCCCCTACT	171	37	One primer sits in the repeat region
BRCA2	BRCA2_Intron_3_4	13	32898184	32898355	AGTGACTAA ACACTGACGACATGGTTCTA CATACTCAGATTGCTCAC	TACGGTAGCAGAGACTTGGTCTT TAAGTGTTCGCCCCCTATGTG	172	40	Two primers sits in the repeat region
BRCA2	BRCA2_Intron_3_5	13	32898267	32898457	GCCTA ACACTGACGACATGGTTCTA CAGGGATCACTACAGTTTC	TACGGTAGCAGAGACTTGGTCTG CTGTTTCCCCTTCTTGC TACGGTAGCAGAGACTTGGTCTA ATTTTATGATGCCATTCTTAC AGTC	191	44	Two primers sits in the repeat region
BRCA2	BRCA2_Intron_3_6	13	32898366	32898559	TGGAGG ACACTGACGACATGGTTCTA CACAGGTCAGAAAAGCAA GAAGGG	TACGGTAGCAGAGACTTGGTCTT TTCTCATTTTAAATTGTGG TACAT	194	38	Two primers sits in the repeat region
BRCA2	BRCA2_Intron_3_7	13	32898426	32898622	ACACTGACGACATGGTTCTA CAAGTAGACTTAAAGAA GGCATCA	TACGGTAGCAGAGACTTGGTCTT GACAATATCATTGTCTAAAC CT	197	30	Two primers sits in the repeat region
BRCA2	BRCA2_Intron_3_8	13	32898527	32898716	ACACTGACGACATGGTTCTA CACCAAAGAATGCAAAATTAT AATCCAGAGT	TACGGTAGCAGAGACTTGGTCTA GCTTCATCACCTTCACTAAGA C	190	27	One primer sits in the repeat region
BRCA2	BRCA2_5	13	32899149	32899334	ACACTGACGACATGGTTCTA CATGATTTCTGTCCACTT CTAAA	TACGGTAGCAGAGACTTGGTCTT CTACCAAGGCTTACGCCAAAT	186	34	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_6	13	32899280	32899435	ACACTGACGACATGGTTCTA CAAAATAACCTAACGGGATT TGCTTGT	TACGGTAGCAGAGACTTGGTCTT GAAACAAACTCCCACATACCACT	156	28	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_7	13	32900202	32900400	ACACTGACGACATGGTTCTA CATGTTAATAAAAAAATAAA TAACAAATTCCCCTT	TACGGTAGCAGAGACTTGGTCTT GAGGCAGAATGCTAGGTACAGA	199	30	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_8	13	32900331	32900527	ACACTGACGACATGGTTCTA CAACGTTAAGTGAATAAAG AGTGAATGAA	TACGGTAGCAGAGACTTGGTCTG GTGGGTGGTAGCTAAAGA	197	33	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_9	13	32900545	32900728	ACACTGACGACATGGTTCTA CATATTTCTTCCCTCCAGG	TACGGTAGCAGAGACTTGGTCTT CAACCTCATCTGCTTCTTCTG	184	37	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_10	13	32900618	32900797	ACACTGACGACATGGTTCTA CATGTGTATGAACTCAAATA GTAGATGT	TACGGTAGCAGAGACTTGGTCTA GCAATTTCACAGCTAATCAATG TC	180	41	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_11	13	32903475	32903668	ACACTGACGACATGGTTCTA ACACTGACGACATGGTTCTA CATGTCAGACAAACAGC	TACGGTAGCAGAGACTTGGTCTG GCCCAAAAGCACAAGTATAATG	194	29	Two primers sits in the repeat region
BRCA2	BRCA2_Intron_8_1	13	32904422	32904620	TCATA ACACTGACGACATGGTTCTA CATGTTCTTACCATAGATGTC GCAGT	TACGGTAGCAGAGACTTGGTCTG GCCCAAAAGCACAAGTATAATG	199	38	Two primers sits in the repeat region
BRCA2	BRCA2_Intron_8_2	13	32904478	32904657	ACACTGACGACATGGTTCTA CATTATACTTGTGCTTGGG	TACGGTAGCAGAGACTTGGTCTT GCTTGTGTTCAAGTCACCCTTA	180	36	Two primers sits in the repeat region
BRCA2	BRCA2_Intron_8_3	13	32904600	32904770	GCCAT	TACGGTAGCAGAGACTTGGTCTA CTTGGGACATGAATCATCCCTT	171	43	Two primers sits in the repeat region

							CATATTGCAGAAGAGTACATTGAACTGCCTGAAAACCAGATGACTATCT TAAAGACCACTCTGAGGAATGCAGAG		
BRCA2	BRCA2_45	13	32911913	32912084	ACACTGACGACATGGTTCTA CAACATTGAAAGTGCTGAA AACCA	TACGGTAGCAGAGACTTGGTCTT TTCAACAGGCCAGCAAACCTC	172	41	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_46	13	32912025	32912218	ACACTGACGACATGGTTCTA CAGCAGCAAGCAATTGAAAG GTACA	TACGGTAGCAGAGACTTGGTCTA CTAAACAGTTCACAGCTTTTC	194	38	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_47	13	32912129	32912305	ACACTGACGACATGGTTCTA CAGAA GTGGGTTAGGGG CTTTA	TACGGTAGCAGAGACTTGGTCTT GAAACAAACAGAAATCATGACATT CTT	177	37	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_48	13	32912216	32912409	ACACTGACGACATGGTTCTA CAAGTGATATTGAGAATTA GTGAGGAAACT	TACGGTAGCAGAGACTTGGTCTA CAAAGTGCAGTAGTCATTCA	194	27	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_49	13	32912285	32912479	ACACTGACGACATGGTTCTA CATGTCATGATTCTGGTTT CATGT	TACGGTAGCAGAGACTTGGTCTT CTACTGGCACAGTATTTGTT	195	26	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_50	13	32912391	32912574	ACACTGACGACATGGTTCTA CATGACTACTGGCAC TTGAAG	TACGGTAGCAGAGACTTGGTCTT GATCGTAATAGCAAGTCGT	184	30	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_51	13	32912431	32912629	ACACTGACGACATGGTTCTA CACAA GAGAAATCTGAAAA TGAAGATAACAAAT	TACGGTAGCAGAGACTTGGTCTC TGAGTGTCCCTCCCTCATAA	199	33	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_52	13	32912534	32912717	ACACTGACGACATGGTTCTA CAGGCCAGTTTGAAGGAG GGAAA	TACGGTAGCAGAGACTTGGTCTT CCCACCTGCGAGTCGAAAAAATG	184	34	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_53	13	32912600	32912792	ACACTGACGACATGGTTCTA CAAGAAGCATGTCA TACTCAA	TACGGTAGCAGAGACTTGGTCTA AAGTTATGCAATTCTCTGGTT T	193	35	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_54	13	32912680	32912872	ACACTGACGACATGGTTCTA CATGGGAAAATTAGTGT CGCCAAAG	TACGGTAGCAGAGACTTGGTCTA CCA CTTGGACACTTCTTCA	193	30	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_55	13	32912788	32912986	ACACTGACGACATGGTTCTA CAACCAAGAAATTGCATAA CTTTCC	TACGGTAGCAGAGACTTGGTCTC TTTCATCACGTCGGGTGTC	199	30	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_56	13	32912851	32913037	ACACTGACGACATGGTTCTA CAAAGAAA GTGCCAGTT GGT	TACGGTAGCAGAGACTTGGTCTT GGT GATTCACTAGTACCTTG	187	36	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_57	13	32912966	32913164	ACACTGACGACATGGTTCTA CATCATACAGCTAGCGGGAA AAAAGT	TACGGTAGCAGAGACTTGGTCTT GATCTCAATGGTCTCACATGCT	199	39	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_58	13	32913064	32913248	ACACTGACGACATGGTTCTA CAAAGACCTAAAGTACAGA GAGGC	TACGGTAGCAGAGACTTGGTCTT GATGTTTGAGATTTCTAGTT CT	185	38	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_59	13	32913185	32913382	ACACTGACGACATGGTTCTA CACCTGTTTCTATTGAGACT GTGGTG	TACGGTAGCAGAGACTTGGTCTT CAATGACTGAATAAGGGACTG	198	37	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_60	13	32913301	32913486	ACACTGACGACATGGTTCTA CAAGACAACTGAAAATCTC AAAACATCAA	TACGGTAGCAGAGACTTGGTCTT GAAGTCTGACTCACAGAAGTT TC	186	33	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_61	13	32913356	32913547	ACACTGACGACATGGTTCTA CATCAGTCATTGAAATTCA GCCTTAGC	TACGGTAGCAGAGACTTGGTCTT CAGCTATAGTACTGTTGAATT TT	192	32	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_62	13	32913476	32913669				31	Assays designed by relax mode and have no off-target hits

BRCA2	BRCA2_83	13	32920895	32921044	ACACTGACGACATGGTTCTA CACTATTACAGTAACATGG ATATTCTCTTACGGTCTA ACACTGACGACATGGTTCTA CATTGTTCCTAGGCACAAAT AAAAGA	TACGGTAGCAGAGACTTGGTCTA CATGTTCTACCGAAGGGTCTA TACGGTAGCAGAGACTTGGTCTT GTTCATTTAAAAACGAGACTTT TC	150	31	CCAGATGGTAAATTAGCTTTTATTTATCTGTTCCCTCTATAGGT TGTTATATAATTatcgac	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_127	13	32920953	32921102	ACACTGACGACATGGTTCTA CACAAATGAGGGTCTGCAAC AAAAG	TACGGTAGCAGAGACTTGGTCTT GCTTGAAGATTTTCCAAGTCAG G	150	31	GTTTTCTCTAGGCCACAATAAAGATCGAAGATGTTTATGCATCA TTAGAGCCGATTACCTGTGACCCCTTCGGTAAGACATGTTAAATT CTAACATTCTAACAGTGTAGAAAAGATCTCGTACCTTAAATT CAAATGGGGTCTGCAACAAAGGCATATTCTAACATTATTTATGTGTC TAGTCATAAACTTATATTTCTCCCATTGCAAGCACAAGTAAAGGAAC GTCAGAAGATACAGAATCCAAATTTCAGCGCAGCTGGTAAGAACATTCTG TCTAACATCTATTGTGATGACATCTGACTTGGAAAATCTTCAGCA GCACAACTAAGGAACGTCAGAGAGATACAGAACATCCAAATTACCGCACC TGGTAGAAGATTCTGCTAAATCTCATTTGTGATGAACTCTGTTGGA AAAATCTTCAAGCAATTCTGAGCTTCCAGGACATTCATTATCAAGTT TGCTACAAGAAATGAAAAATGAGACACTTGATTACTACAGGCAGACC TTAGCGATTTCAGGACACCATTTTCAAGTGTGAGGAAATGAG AAAATGAGGACACTTGATTACTACAGGCAGACCAAAAGTCTTGGTC CACCTTTAAACTAAATCACATTTCACAGAGTTGAAACAGTGTGTTAGG AATTTAACATTGGAGAACACGACA TACTACAGGAGAACACAAAGTCTTGTCCACCTTTAAACTAAAT CACATTTCACAGAGTTGAACTGAGCTGTTAGGAATTTAACTGGAGGA AACAGACAAAAGAACAAAGGACATGGACATGGCTCTGTGATGAACTTAA TAAGATTAACTGACAATGAGATTCTACATGTTAACAAAACAACTCCA CTGATGATAGTAAATAAAGATTAATGACAATGAGATTCTACAGTAA AAAACAATCCAACATAGCAGTAGCTGTAACCTTCAACAAAGTGTGAA GAACCTTTAGGTATTGTGATGACAAATTGTGATGAAATTGCGTTTACAG ggccAGGGGGTGTGCTTTAAATTCAATTATTGCTAAGTATT CTTGATAGTTAACTACAAGTCTTCAGAATGCCAGAGATAACAGGAT ATGCGAAATAAGAAGAACAAAGGCAACGCGCTTCCACAGGCCAGGCA GTCTGTATCTGCAAAACATCTCCTGCGCTCG TGCAGATTAAAGAACAAAGGCAACGCGCTTCCACAGGCCAGGAG TCTGTATCTGCAAAACATCTCCTGCGCTCG CAGTAGGAGGCCAAGTCCCTCGCGTCTCATAAAACAGGTATGTG TTGCTACAACTACTGATGCTTTATGACAGAGTGT TTGTTTATTGTGATACATGTTACTTAAATTGTTTCTTTGT TGTGTTTATTGTGATGCTGTTACGTTGCGTCTCATAACATTGCA AAAATTAACAGCAAAATGAGCTTTCAGTTACACTGAA TTTGGTAAGGAAAGTTGAGCTGGAAAAGGAATACAGTGGCT GCATAAAAATTAACAGCAAAATGAGCTTTCAGTTACACTGAA GATTATTGGTAAGGAAAGTTGACTGGAAAAGGAATACAGTGGC TGATGGGGAGCTGCATACCCCTCAAGTGGAAAGCTGGAAAAGGA GAATTATTAGGTACTCTGCAAAAGGATGTTGAACTT TGTACAGGAAATAGTTGAGTTGTTGAATTCACTATCATCTATG TTTATGATAATTCTACTTTATTGTGAGCTGCTCTGTGACACTCCA GGTGTGAGTCAAAGCTTATTCTAGAATTGGTTATACTACATAG ATGGATCATATGGAAACTGGCAGCTATGGAATGTCCTTCC GCTCTGTGAGCACTCAGGGTGGCAAAGCTTCTAGAATT GGTTATAAACTACTATAGGATCATATGGAAACTGGCAGCTATG GTGCTTCTCTAAGGAATTGCTAATAGATGCCTAAGGCCAGAAAGGG GCTCTCTCAACAAATACAG ATGGAACACTGGCAGCTATGGAATGTCCTTCC GATGCCCTAAGCCCAGAAAGGGTCTTCAAGGAATTGCTA GATGCCCTAAGCCCAGAAAGGGTCTTCAACTAAACACGGCAAGT TTAACGATTACCTTACGCTAATCATGCGCAGTATGTTAAGGTTCT GTGAGTCTGTGACTCTCAGTCAGGAACTTGTGCAACAGCAGTGT TGAATCTCTAGAGTCACACTCTCCTAAATATGCATTTGTTTACTT AGATATGATGACGGAAATTGATGAAAGCAGAAAGTCGCTATAAAAGAT AATGGAAGGGATGACACAGCTGCAAAACACTGTTCTGTGTTCTG ACATAATTCTTACAGCGCA TGGAAAGGGATGACACAGCTGCAAAACACTGTTCTGTGTTCTG CATAATTCTTACAGCGCAAAATATGCAACTTCTGCAAAACTAG TAGTCAGAGTACCCAAAAGTGGCCATTATGAACTTACAGATGGG TATGCTGTTAAGGCCAGTTAGATCTCCCTCTAGCTGCTTAAAGA GTAGTCAGACATGGCCAGTTAGATCTCCCTCTAGCTGCTTAAAGA ATGGCAGACTGGCAGCTGGTCAAGAGATCTCTGTTCTG TTAGGAGACTGAGCTGGTCAAGAGATCTCTGTTCTG GGTGGGCTCTCTGATCTGACACCTCTGAG TTCTTCATGGAGCAGAACCTGGGGCTCTGATGCGCTGACACCT TGAAGCCCGAACATCTTGTGAAAGGAAATTAACTGACTCTGG TAAACATGCTGTTAGGCTTACAGTCAAGTGGG TAAATGCTTACTAAGGATGCTCAATTCTTAGATGACTGA TTTTAAAGTGAATTGTTAAGGCAGTTCTAGAAGAATGAAAACCTT GATATCTGTAATAGAATTGAAACATATTAACTACTAAATCAATATATT	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_85	13	32928912	32929110	ACACTGACGACATGGTTCTA CACAAATGAGGGTCTGCAAC AAAAG	TACGGTAGCAGAGACTTGGTCTT GCTTGAAGATTTTCCAAGTCAG G	199	35	CTAACATTCTAACAGTGTAGAAAAGATCTCGTACCTT CAAATGGGGTCTGCAACAAAGGCTATTCTGAGCTAACATT GTCAGAAGATACAGAATCCAAATTTCAGCGCAGCTGGTAAGAACATT TCTAACATCTATTGTGATGACATCTGACTTGGAAAATCTTCAGCA GCACAACTAAGGAACGTCAGAGAGATACAGAACATCCAAATTACCGCACC TGGTAGAAGATTCTGCTAAATCTCATTTGTGATGAACTCTGTTGGA AAAATCTTCAAGCAATTCTGAGCTTCCAGGACATTCATTATCAAGTT TGCTACAAGAAATGAAAAATGAGACACTTGATTACTACAGGCAGACC TTAGCGATTTCAGGACACCATTTTCAAGTGTGAGGAAATGAG AAAATGAGGACACTTGATTACTACAGGCAGACCAAAAGTCTTGGTC CACCTTTAAACTAAATCACATTTCACAGAGTTGAAACAGTGTGTTAGG AATTTAACATTGGAGAACACGACA TACTACAGGAGAACACAAAGTCTTGTCCACCTTTAAACTAAAT CACATTTCACAGAGTTGAACTGAGCTGTTAGGAATTTAACTGGAGGA AACAGACAAAAGAACAAAGGACATGGACATGGCTCTGTGATGAACTTAA TAAGATTAACTGACAATGAGATTCTACATGTTAACAAAACAACTCCA CTGATGATAGTAAATAAAGATTAATGACAATGAGATTCTACAGTAA AAAACAATCCAACATAGCAGTAGCTGTAACCTTCAACAAAGTGTGAA GAACCTTTAGGTATTGTGATGACAAATTGTGATGAAATTGCGTTTACAG T	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_86	13	32928997	32929195	ACACTGACGACATGGTTCTA CACGACAACTAAGAACGTC AAGAG	TACGGTAGCAGAGACTTGGTCTG GTCTGCCTGTAGTAATCAAGTG	199	36	AAATCTTCAAGCAATTCTGAGCTTCCAGGACATTCATTATCAAGTT TGCTACAAGAAATGAAAAATGAGACACTTGATTACTACAGGCAGACC TTAGCGATTTCAGGACACCATTTTCAAGTGTGAGGAAATGAG AAAATGAGGACACTTGATTACTACAGGCAGACCAAAAGTCTTGGTC CACCTTTAAACTAAATCACATTTCACAGAGTTGAAACAGTGTGTTAGG AATTTAACATTGGAGAACACGACA TACTACAGGAGAACACAAAGTCTTGTCCACCTTTAAACTAAAT CACATTTCACAGAGTTGAACTGAGCTGTTAGGAATTTAACTGGAGGA AACAGACAAAAGAACAAAGGACATGGACATGGCTCTGTGATGAACTTAA TAAGATTAACTGACAATGAGATTCTACATGTTAACAAAACAACTCCA CTGATGATAGTAAATAAAGATTAATGACAATGAGATTCTACAGTAA AAAACAATCCAACATAGCAGTAGCTGTAACCTTCAACAAAGTGTGAA GAACCTTTAGGTATTGTGATGACAAATTGTGATGAAATTGCGTTTACAG T	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_87	13	32929113	32929288	ACACTGACGACATGGTTCTA CATTAGCAGTTTCAGGACAT CCATT	TACGGTAGCAGAGACTTGGTCTT GTCTGTTTCCCTCCAAGTTAAT TCC	176	35	AAATCTTCAAGCAATTCTGAGCTTCCAGGACATTCATTATCAAGTT TGCTACAAGAAATGAAAAATGAGACACTTGATTACTACAGGCAGACC TTAGCGATTTCAGGACACCATTTTCAAGTGTGAGGAAATGAG AAAATGAGGACACTTGATTACTACAGGCAGACCAAAAGTCTTGGTC CACCTTTAAACTAAATCACATTTCACAGAGTTGAAACAGTGTGTTAGG AATTTAACATTGGAGAACACGACA TACTACAGGAGAACACAAAGTCTTGTCCACCTTTAAACTAAAT CACATTTCACAGAGTTGAACTGAGCTGTTAGGAATTTAACTGGAGGA AACAGACAAAAGAACAAAGGACATGGACATGGCTCTGTGATGAACTTAA TAAGATTAACTGACAATGAGATTCTACATGTTAACAAAACAACTCCA CTGATGATAGTAAATAAAGATTAATGACAATGAGATTCTACAGTAA AAAACAATCCAACATAGCAGTAGCTGTAACCTTCAACAAAGTGTGAA GAACCTTTAGGTATTGTGATGACAAATTGTGATGAAATTGCGTTTACAG T	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_88	13	32929181	32929377	ACACTGACGACATGGTTCTA CATACTACAGGCAACCAAC CAAAAG	TACGGTAGCAGAGACTTGGTCTT GGAGTTTTTGTAACTGATG A	197	34	AAATCTTCAAGCAATTCTGAGCTTCCAGGACATTCATTATCAAGTT TGCTACAAGAAATGAAAAATGAGACACTTGATTACTACAGGCAGACC TTAGCGATTTCAGGACACCATTTTCAAGTGTGAGGAAATGAG AAAATGAGGACACTTGATTACTACAGGCAGACCAAAAGTCTTGGTC CACCTTTAAACTAAATCACATTTCACAGAGTTGAAACAGTGTGTTAGG AATTTAACATTGGAGAACACGACA TACTACAGGAGAACACAAAGTCTTGTCCACCTTTAAACTAAAT CACATTTCACAGAGTTGAACTGAGCTGTTAGGAATTTAACTGGAGGA AACAGACAAAAGAACAAAGGACATGGACATGGCTCTGTGATGAACTTAA TAAGATTAACTGACAATGAGATTCTACATGTTAACAAAACAACTCCA CTGATGATAGTAAATAAAGATTAATGACAATGAGATTCTACAGTAA AAAACAATCCAACATAGCAGTAGCTGTAACCTTCAACAAAGTGTGAA GAACCTTTAGGTATTGTGATGACAAATTGTGATGAAATTGCGTTTACAG T	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_89	13	32929315	32929467	ACACTGACGACATGGTTCTA CACTGATGATACTAAAATAA GATTAATGACAATGAG	TACGGTAGCAGAGACTTGGTCTA CTGAAAGCAAAATTCTCACA CA	153	31	ggccAGGGGGTGTGCTTTAAATTCAATTATTGCTAAGTATT CTTGATAGTTAACTACAAGTCTTCAGAATGCCAGAGATAACAGGAT ATGCGAAATAAGAAGAACAAAGGCAACCGCTTCCACAGGCCAGGCA GTCTGTATCTGCAAAACATCTCCTGCGCTCG TGCAGATTAAAGAACAAAGGCAACCGCTTCCACAGGCCAGGAG TCTGTATCTGCAAAACATCTCCTGCGCTCG CAGTAGGAGGCCAAGTCCCTCGCGTCTCATAAAACAGGTATGTG TTGCTACAACTACTGATGCTTTATGACAGAGTGT TTGTTTATTGTGATACATGTTACTTAAATTGTTTCTTTGT TGTGTTTATTGTGATGCTGTTACGTTGAGCTGGTTCTCATAACATTG AAAATTAACAGCAAAATGAGCTTTCAGTTACCTGAA TTTGGTAAGGAAAGTTGAGCTGGAAAAGGAATACAGTGGCT GCATAAAAATTAACAGCAAAATGAGCTTTCAGTTACACTGAA GATTATTGGTAAGGAAAGTTTATGACTGGACTGGAAAAGGAATACAGTGGC TGATGGGGAGCTGCATACCCCTCAAGTGGAAAGCTGGAAAAGGA GAATTTTATAGGTACTCTGCAAAAGGATGTTGAACTT TGTACAGGAAATAGTTGAGTTGTTGAATTCACTATCATCTATG TTTATGATAATTCTACTTTATTGTGAGCTGGCTCTGTGACACTCCA GGTGTGAGTCAAAGCTTATTCTAGAATTGGTTATACTACATAG ATGGATCATATGGAAACTGGCAGCTATGGAATGTCCTTCC GCTCTGTGAGCACTCAGGGTGGCTTACAGCTGGCTGGAAAGCT GGTTATAAACTACTATAGGATGATCATGGAAACTGGCAGCTATGG GTGCTTCTCTAAGGAATTGCTAATAGATGCCTAAGGCCAGAAAGGG GCTCTCTCAACAAATACAG ATGGAACACTGGCAGCTATGGAATGTCCTTCC GATGCCCTAAGCCCAGAAAGGGTCTTCAAGGAATTGCTA GATGCCCTAAGCCCAGAAAGGGTCTTCAACTAAACACGGCAAGT TTAACGATTACCTTACGCTAATCATGCGCAGTATGTTAAGGTTCT GTGAGTCTGTGACTCTCAGTCAGGAACTTGTGCAACAGCAGTGT TGAATCTCTAGAGTCACACTCTCCTAAATATGCATTTGTTTACTT AGATATGATGACGGAAATTGATGAAAGCAGAAAGTCGCTATAAAAGAT AATGGAAGGGATGACACAGCTGCAAAACACTGTTCTGTGTTCTG ACATAATTCTTACAGCGCA TGGAAAGGGATGACACAGCTGCAAAACACTGTTCTGTGTTCTG CATAATTCTTACAGCGCAAAATATGCAACTTCTGCAAAACTAG TAGTCAGAGTACCCAAAAGTGGCCATTATGAACTTACAGATGGG TATGCTGTTAAGGCCAGTTAGATCTCCCTCTAGCTGCTTAAAGA GTAGTCAGACATGGCCAGTTAGATCTCCCTCTAGCTGCTTAAAGA ATGGCAGACTGAGCTGGTCAAGAGATCTCTGTTCTG TTAGGAGACTGAGCTGGTCAAGAGATCTCTGTTCTG GGTGGGCTCTCTGATCTGACACCTCTGAG TTCTTCATGGAGCAGAACCTGGGGCTCTGATGCGCTGACACCT TGAAGCCCGAACATCTTGTGAAAGGAAATTAACTGACTCTGG TAAACATGCTGTTAGGCTTACAGTCAAGTAACTGAGTTTACATT TAAATGCTTACTAAGGATGCTCAATTCTTAGATGACTGA TTTTAAAGTGAATTGTTAAGGCAGTTCTAGAAGAATGAAAACCTT GATATCTGTAATAGAATTGAAACATATTAACTACTAAATCAATATATT	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_90	13	32930503	32930690	ACACTGACGACATGGTTCTA CAGGCCAGGGTTGTGCTT TTA	TACGGTAGCAGAGACTTGGTCTT TCGAGGCGAGAGTGGATGTTT	188	37	AAATCTTCAAGCAATTCTGAGCTTCCAGGACATTCATTATCAAGTT TGCTACAAGAAATGAAAAATGAGACACTTGATTACTACAGGCAGACC TTAGCGATTTCAGGACACCATTTTCAAGTGTGAGGAAATGAG AAAATGAGGACACTTGATTACTACAGGCAGACCAAAAGTCTTGGTC CACCTTTAAACTAAATCACATTTCACAGAGTTGAAACAGTGTGTTAGG AATTTAACATTGGAGAACACGACA TACTACAGGAGAACACAAAGTCTTGTCCACCTTTAAACTAAAT CACATTTCACAGAGTTGAACTGAGCTGTTAGGAATTTAACTGGAGGA AACAGACAAAAGAACAAAGGACATGGACATGGCTCTGTGATGAACTTAA TAAGATTAACTGACAATGAGATTCTACATGTTAACAAAACAACTCCA CTGATGATAGTAAATAAAGATTAATGACAATGAGATTCTACAGTAA AAAACAATCCAACATAGCAGTAGCTGTAACCTTCAACAAAGTGTGAA GAACCTTTAGGTATTGTGATGACAAATTGTGATGAAATTGCGTTTACAG T	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_91	13	32930607	32930790	ACACTGACGACATGGTTCTA CATCGCAATTAAAGAAGAAC AAAGC	TACGGTAGCAGAGACTTGGTCTA CACTCTGTCTAAAGGCTAC G	184	45	AAATCTTCAAGCAATTCTGAGCTTCCAGGACATTCATTATCAAGTT TGCTACAAGAAATGAAAAATGAGACACTTGATTACTACAGGCAGACC TTAGCGATTTCAGGACACCATTTTCAAGTGTGAGGAAATGAG AAAATGAGGACACTTGATTACTACAGGCAGACCAAAAGTCTTGGTC CACCTTTAAACTAAATCACATTTCACAGAGTTGAAACAGTGTGTTAGG AATTTAACATTGGAGAACACGACA TACTACAGGAGAACACAAAGTCTTGTCCACCTTTAAACTAAAT CACATTTCACAGAGTTGAACTGAGCTGTTAGGAATTTAACTGGAGGA AACAGACAAAAGAACAAAGGACATGGACATGGCTCTGTGATGAACTTAA TAAGATTAACTGACAATGAGATTCTACATGTTAACAAAACAACTCCA CTGATGATAGTAAATAAAGATTAATGACAATGAGATTCTACAGTAA AAAACAATCCAACATAGCAGTAGCTGTAACCTTCAACAAAGTGTGAA GAACCTTTAGGTATTGTGATGACAAATTGTGATGAAATTGCGTTTACAG T	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_92	13	32931809	32932007	ACACTGACGACATGGTTCTA CATTGTTTATTGTGTTGATA CATGTTTACTTT	TACGGTAGCAGAGACTTGGTCTA GCCAACTGTATTCTTTCCAGT	199	30	AAATCTTCAAGCAATTCTGAGCTTCCAGGACATTCATTATCAAGTT TGCTACAAGAAATGAAAAATGAGACACTTGATTACTACAGGCAGACC TTAGCGATTTCAGGACACCATTTTCAAGTGTGAGGAAATGAG AAAATGAGGACACTTGATTACTACAGGCAGACCAAAAGTCTTGGTC CACCTTTAAACTAAATCACATTTCACAGAGTTGAAACAGTGTGTTAGG AATTTAACATTGGAGAACACGACA TACTACAGGAGAACACAAAGTCTTGTCCACCTTTAAACTAAAT CACATTTCACAGAGTTGAACTGAGCTGTTAGGAATTTAACTGGAGGA AACAGACAAAAGAACAAAGGACATGGACATGGCTCTGTGATGAACTTAA TAAGATTAACTGACAATGAGATTCTACATGTTAACAAAACAACTCCA CTGATGATAGTAAATAAAGATTAATGACAATGAGATTCTACAGTAA AAAACAATCCAACATAGCAGTAGCTGTAACCTTCAACAAAGTGTGAA GAACCTTTAGGTATTGTGATGACAAATTGTGATGAAATTGCGTTTACAG T	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_93	13	32931907	32932099	ACACTGACGACATGGTTCTA CACCATAAAATTAAACAGCA AAAATGCA	TACGGTAGCAGAGACTTGGTCTA AAGTTAACACACAACTTTTGCA TAG	193	36	AAATCTTCAAGCAATTCTGAGCTTCCAGGACATTCATTATCAAGTT TGCTACAAGAAATGAAAAATGAGACACTTGATTACTACAGGCAGACC TTAGCGATTTCAGGACACCATTTTCAAGTGTGAGGAAATGAG AAAATGAGGACACTTGATTACTACAGGCAGACCAAAAGTCTTGGTC CACCTTTAAACTAAATCACATTTCACAGAGTTGAAACAGTGTGTTAGG AATTTAACATTGGAGAACACGACA TACTACAGGAGAACACAAAGTCTTGTCCACCTTTAAACTAAAT CACATTTCACAGAGTTGAACTGAGCTGTTAGGAATTTAACTGGAGGA AACAGACAAAAGAACAAAGGACATGGACATGGCTCTGTGATGAACTTAA TAAGATTAACTGACAATGAGATTCTACATGTTAACAAAACAACTCCA CTGATGATAGTAAATAAAGATTAATGACAATGAGATTCTACAGTAA AAAACAATCCAACATAGCAGTAGCTGTAACCTTCAACAAAGTGTGAA GAACCTTTAGGTATTGTGATGACAAATTGTGATGAAATTGCGTTTACAG T	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_94	13	32936578	32936771	ACACTGACGACATGGTTCTA CATGTACAGAGAAATGTTG AGTTGTTGA	TACGGTAGCAGAGACTTGGTCTC GGAAAGGCACATTCCATAGCTG	194	36	AAATCTTCAAGCAATTCTGAGCTTCCAGGACATTCATTATCAAGTT TGCTACAAGAAATGAAAAATGAGACACTTGATTACTACAGGCAGACC TTAGCGATTTCAGGACACCATTTTCAAGTGTGAGGAAATGAG AAAATGAGGACACTTGATTACTACAGGCAGACCAAAAGTCTTGGTC CACCTTTAAACTAAATCACATTTCACAGAGTTGAAACAGTGTGTTAGG AATTTAACATTGGAGAACACGACA TACTACAGGAGAACACAAAGTCTTGTCCACCTTTAAACTAAAT CACATTTCACAGAGTTGAACTGAGCTGTTAGGAATTTAACTGGAGGA AACAGACAAAAGAACAAAGGACATGGACATGGCTCTGTGATGAACTTAA TAAGATTAACTGACAATGAGATTCTACATGTTAACAAAACAACTCCA CTGATGATAGTAAATAAAGATTAATGACAATGAGATTCTACAGTAA AAAACAATCCAACATAGCAGTAGCTGTAACCTTCAACAAAGTGTGAA GAACCTTTAGGTATTGTGATGACAAATTGTGATGAAATTGCGTTTACAG T	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_95	13	32936661	32936831	ACACTGACGACATGGTTCTA CAGCTCTGTGTGACACTCCA GGT	TACGGTAGCAGAGACTTGGTCTC CTGTATTTTAGTTGAGAAGC AC	171	41	AAATCTTCAAGCAATTCTGAGCTTCCAGGACATTCATTATCAAGTT TGCTACAAGAAATGAAAAATGAGACACTTGATTACTACAGGCAGACC TTAGCGATTTCAGGACACCATTTTCAAGTGTGAGGAAATGAG AAAATGAGGACACTTGATTACTACAGGCAGACCAAAAGTCTTGGTC CACCTTTAAACTAAATCACATTTCACAGAGTTGAAACAGTGTGTTAGG AATTTAACATTGGAGAACACGACA TACTACAGGAGAACACAAAGTCTTGTCCACCTTTAAACTAAAT CACATTTCACAGAGTTGAACTGAGCTGTTAGGAATTTAACTGGAGGA AACAGACAAAAGAACAAAGGACATGGACATGGCTCTGTGATGAACTTAA TAAGATTAACTGACAATGAGATTCTACATGTTAACAAAACAACTCCA CTGATGATAGTAAATAAAGATTAATGACAATGAGATTCTACAGTAA AAAACAATCCAACATAGCAGTAGCTGTAACCTTCAACAAAGTGTGAA GAACCTTTAGGTATTGTGATGACAAATTGTGATGAAATTGCGTTTACAG T	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_96	13	32936738	32936933	ACACTGACGACATGGTTCTA CAATGGAAACTGGCAGCTAT GGAAT	TACGGTAGCAGAGACTTGGTCTG ACAATGGCTTGTGACACATT	196	40	AAATCTTCAAGCAATTCTGAGCTTCCAGGACATTCATTATCAAGTT TGCTACAAGAAATGAAAAATGAGACACTTGATTACTACAGGCAGACC TTAGCGATTTCAGGACACCATTTTCAAGTGTGAGGAAATGAG AAAATGAGGACACTTGATTACTACAGGCAGACCAAAAGTCTTGGTC CACCTTTAAACTAAATCACATTTCACAGAGTTGAAACAGTGTGTTAGG AATTTAACATTGGAGAACACGACA TACTACAGGAGAACACAAAGTCTTGTCCACCTTTAAACTAAAT CACATTTCACAGAGTTGAACTGAGCTGTTAGGAATTTAACTGGAGGA AACAGACAAAAGAACAAAGGACATGGACATGGCTCTGTGATGAACTTAA TAAGATTAACTGACAATGAGATTCTACATGTTAACAAAACAACTCCA CTGATGATAGTAAATAAAGATTAATGACAATGAGATTCTACAGTAA AAAACAATCCAACATAGCAGTAGCTGTAACCTTCAACAAAGTGTGAA GAACCTTTAGGTATTGTGATGACAAATTGTGATGAAATTGCGTTTACAG T	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_97	13	32937263	32937433	ACACTGACGACATGGTTCTA CATGGAATTCTTAGAGTC CTTCTAA	TACGGTAGCAGAGACTTGGTCTT GCGCTCAATGAAATTATGTCAG AAC	171	35	AAATCTTCAAGCAATTCTGAGCTTCCAGGACATTCATTATCAAGTT TGCTACAAGAAATGAAAAATGAGACACTTGATTACTACAGGCAGACC TTAGCGATTTCAGGACACCATTTTCAAGTGTGAGGAAATGAG AAAATGAGGACACTTGATTACTACAGGCAGACCAAAAGTCTTGGTC CACCTTTAAACTAAATCACATTTCACAGAGTTGAAACAGTGTGTTAGG AATTTAACATTGGAGAACACGACA TACTACAGGAGAACACAAAGTCTTGTCCACCTTTAAACTAAAT CACATTTCACAGAGTTGAACTGAGCTGTTAGGAATTTAACTGGAGGA AACAGACAAAAGAACAAAGGACATGGACATGGCTCTGTGATGAACTTAA TAAGATTAACTGACAATGAGATTCTACATGTTAACAAAACAACTCCA CTGATGATAGTAAATAAAGATTAATGACAATGAGATTCTACAGTAA AAAACAATCCAACATAGCAGTAGCTGTAACCTTCAACAAAGTGTGAA GAACCTTTAGGTATTGTGATGACAAATTGTGATGAAATTGCGTTTACAG T	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_98	13	32937366	32937563	ACACTGACGACATGGTTCTA CATGAAAGGGATGACACAG C	TACGGTAGCAGAGACTTGGTCTT CTTAAAGACAGCTAAGGGGGAG	198	40	AAATCTTCAAGCAATTCTGAGCTTCCAGGACATTCATTATCAAGTT TGCTACAAGAAATGAAAAATGAGACACTTGATTACTACAGGCAGACC TTAGCGATTTCAGGACACCATTTTCAAGTGTGAGGAAATGAG AAAATGAGGACACTTGATTACTACAGGCAGACCAAAAGTCTTGGTC CACCTTTAAACTAAATCACATTTCACAGAGTTGAAACAGTGTGTTAGG AATTTAACATTGGAGAACACGACA TACTACAGGAGAACACAAAGTCTTGTCCACCTTTAAACTAAAT CACATTTCACAGAGTTGAACTGAGCTGTTAGGAATTTAACTGGAGGA AACAGACAAAAGAACAAAGGACATGGACATGGCTCTGTGATGAACTTAA TAAGATTAACTGACAATGAGATTCTACATGTTAACAAAACAACTCCA CTGATGATAGTAAATAAAGATTAATGACAATGAGATTCTACAGTAA AAAACAATCCAACATAGCAGTAGCTGTAACCTTCAACAAAGTGTGAA GAACCTTTAGGTATTGTGATGACAAATTGTGATGAAATTGCGTTTACAG T	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_99	13	32937465	32937648	ACACTGACGACATGGTTCTA CACTAGTCAGCATACCCAAA AAGTGG	TACGGTAGCAGAGACTTGGTCTG CTTCAAGAGGTGTACAGGCATC	184	47	AAATCTTCAAGCAATTCTGAGCTTCCAGGACATTCATTATCAAGTT TGCTACAAGAAATGAAAAATGAGACACTTGATTACTACAGGCAGACC TTAGCGATTTCAGGACACCATTTTCAAGTGTGAGGAAATGAG AAAATGAGGACACTTGATTACTACAGGCAGACCAAAAGTCTTGGTC CACCTTTAAACTAAATCACATTTCACAGAGTTGAAACAGTGTGTTAGG AATTTAACATTGGAGAACACGACA TACTACAGGAGAACACAAAGTCTTGTCCACCTTTAAACTAAAT CACATTTCACAGAGTTGAACTGAGCTGTTAGGAATTTAACTGGAGGA AACAGACAAAAGAACAAAGGACATGGACATGGCTCTGTGATGAACTTAA TAAGATTAACTGACAATGAGATTCTACATGTTAACAAAACAACTCCA CTGATGATAGTAAATAAAGATTAATGACAATGAGATTCTACAGTAA AAAACAATCCAACATAGCAGTAGCTGTAACCTTCAACAAAGTGTGAA GAACCTTTAGGTATTGTGATGACAAATTGTGATGAAATTGCGTTTACAG T	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_100	13	32937594	32937785	ACACTGACGACATGGTTCTA CATTCTTCATGGAGCAGAC TGGT	TACGGTAGCAGAGACTTGGTCTT CAGTACATCTAAGAAATTGAGCA TCC	192	35	AAATCTTCAAGCAATTCTGAGCTTCCAGGACATTCATTATCAAGTT TGCTACAAGAAATGAAAAATGAGACACTTGATTACTACAGGCAGACC TTAGCGATTTCAGGACACCATTTTCAAGTGTGAGGAAATGAG AAAATGAGGACACTTGATTACTACAGGCAGACCAAAAGTCTTGGTC CACCTTTAAACTAAATCACATTTCACAGAGTTGAAACAGTGTGTTAGG AATTTAACATTGGAGAACACGACA TACTACAGGAGAACACAAAGTCTTGTCCACCTTTAAACTAAAT CACATTTCACAGAGTTGAACTGAGCTGTTAGGAATTTAACTGGAGGA AACAGACAAAAGAACAAAGGACATGGACATGGCTCTGTGATGAACTTAA TAATGCTTACTAAGGATGCTCAATTCTTAGATGACTGA TTTTAAAGTGAATTTTAAGGCAGTTCTAGAAGAATGAAAACACTT GATATCTGTAATAGAATTGAAACATATTAACTACTAAATCAATATATT	Assays designed by relax mode and have no off-target hits
BRCA2	BRCA2_101	13	32944423	32944616	ACACTGACGACATGGTTCTA CAAGGCAAGGTCT TAAGGCAGTTCT	TACGGTAGCAGAGACTTGGTCTC AGAGGAAAAGGTCTAGGGTCA AG	194	32	TTTTAAAGTGAATTTTAAGGCAGTTCTAGAAGAATGAAAACACTT GATATCTGTAATAGAATTGAAACATATTAACTACTAAATCAATATATT	Assays designed by relax mode and have no off-target hits

							TTTCTAGACTAAATACAGTGTGGGAATACACAATAACACAACCTACTAGCC	
							TATGTGAAACACCGAAAGGCCAGAATGAGGAAGTGCTGGAGAACT	
							TAGCCTATGTGAAACACCGAAAGGCCAGAATGAGGAAGTGCTGGAGAACT	
							ACTTGAAGAACAGCTGAAGACTTAATCAGAAAAGAGATGCCAATCAGATT	
							TGAGAAGCCTGTAACCTGGGCAACTTGCCTGGGTATTACACAT	
							GGGCAGACTGGCAGAACCCAGACTTACCTGACAAAGGTAGAGAACAT	
							TTGC	
BRCA2	BRCA2_Intron_24_regi_on_2_3	13	32958974	32959172	ACACTGACGACATGGTTCTA CATAGCTATGTGAAACACC CGAAA	TACGGTAGCAGAGACTTGGTCTG CAAATGTTCTCACCTTGTCCA	199	47
BRCA2	BRCA2_Intron_24_regi_on_2_4	13	32959058	32959234	ACACTGACGACATGGTTCTA CAATGCCAATCAGATTGAG AAGCC	TACGGTAGCAGAGACTTGGTCTT CTTCCTCACAGTCCATCTCTGG	177	47
BRCA2	BRCA2_Intron_24_regi_on_2_5	13	32959147	32959333	ACACTGACGACATGGTTCTA CAACCTGGACAAGGTAGAGA ACATT	TACGGTAGCAGAGACTTGGTCTG TGTGAGTTCAGGGTTTCAGC	187	48
BRCA2	BRCA2_Intron_24_regi_on_2_6	13	32959205	32959401	ACACTGACGACATGGTTCTA CAGGAATGTCAGAGATGGA CTGTG	TACGGTAGCAGAGACTTGGTCTA AAATGACTGTGATCCCCGTT	197	50
BRCA2	BRCA2_Intron_24_regi_on_2_7	13	32959311	32959504	ACACTGACGACATGGTTCTA CAGCTGAAAACCCTGAAC AACAC	TACGGTAGCAGAGACTTGGTCTC CTTCAGCTCCTGTCCTTCATC	194	47
BRCA2	BRCA2_Intron_3_1	13	32964498	32964682	ACACTGACGACATGGTTCTA CAGCGCTCGCAAGGCTA ACACTGACGACATGGTTCTA	TACGGTAGCAGAGACTTGGTCTA CAAAGAAGATTTAAAGGTAG AAGCA	185	32
BRCA2	BRCA2_Intron_3_2	13	32964614	32964772	ACACTGACGACATGGTTCTA CAACTGTACAAGTCATATT CTGATGC	TACGGTAGCAGAGACTTGGTCTA AGCATCTGACTAGGGAGGTAA	159	35
BRCA2	BRCA2_Intron_3_3	13	32964707	32964900	ACACTGACGACATGGTTCTA CACCCCTCTGATGAATT GTCTT	TACGGTAGCAGAGACTTGGTCTA CTGAGGCTAGTAGGTGGCTT	194	44
BRCA2	BRCA2_Intron_3_4	13	32964809	32965000	ACACTGACGACATGGTTCTA ACACTGACGACATGGTTCTA CATGATCTTCAGAACAG	TACGGTAGCAGAGACTTGGTCTC CTGGAGTTTCAACAAGTAC	192	44
BRCA2	BRCA2_Intron_3_5	13	32964908	32965094	GGACT ACACTGACGACATGGTTCTA CACGGTCCATAGTCACAC	TACGGTAGCAGAGACTTGGTCTT AACCTCTGAGCTCCAGTT	187	44
BRCA2	BRCA2_Intron_3_6	13	32965017	32965207	ACACTGACGACATGGTTCTA CATTACAGATGGGAAACT TGTT	TACGGTAGCAGAGACTTGGTCTT GAGTTGCTTTGTTCAGC	191	40
BRCA2	BRCA2_Intron_3_7	13	32965060	32965244	GGAGC ACAGATGTCCTGTAAAGCC	TACGGTAGCAGAGACTTGGTCTT ACAGATGTCCTGTAAAGCC	185	39
BRCA2	BRCA2_Intron_3_8	13	32965183	32965359	ACACTGACGACATGGTTCTA CAtgctgACAAAACAAAAGCA ACT	TACGGTAGCAGAGACTTGGTCT aaACAGGTTTCCCAAACAA	177	34
BRCA2	BRCA2_Intron_3_9	13	32965220	32965418	ACACTGACGACATGGTTCTA CATGGCTTAAACAGGGAC ATCTG	TACGGTAGCAGAGACTTGGTCTG CTGTTCTAACGGCATTTC	199	36
BRCA2	BRCA2_Intron_3_10	13	32965331	32965527	ACACTGACGACATGGTTCTA AAGCC	TACGGTAGCAGAGACTTGGTCTC AGGCTAGCAAGACTCTGAAGG	197	47
BRCA2	BRCA2_Intron_3_11	13	32965437	32965630	AAG ACACTGACGACATGGTTCTA CAAAAGAGCCAGTCCTGC TACT	TACGGTAGCAGAGACTTGGTCTC TCTGAGTCCCTCTGGACTT	194	56
BRCA2	BRCA2_Intron_3_12	13	32965531	32965725	ACACTGACGACATGGTTCTA CACTGGAGGATCACATGC TGG	TACGGTAGCAGAGACTTGGTCTC TGGAGTGGATCCATGGT	195	56
BRCA2	BRCA2_Intron_3_13	13	32965663	32965858	ACACTGACGACATGGTTCTA CACTGGAGGATCACATGC TGG	TACGGTAGCAGAGACTTGGTCTT ATTTCAGCTTTGTGGTCTG	196	43
BRCA2	BRCA2_113	13	32968741	32968935	ACACTGACGACATGGTTCTA CAAGGCATATTAGAGTTCC TTCTTGC	TACGGTAGCAGAGACTTGGTCTT GCAGCAATTACATAGGGCTT	195	32
BRCA2	BRCA2_114	13	32968830	32969028	ACACTGACGACATGGTTCTA CATGCCCTTCTGCTCTATT GTCAG	TACGGTAGCAGAGACTTGGTCTG CCCTTTGGACTAGCAGAA	199	40

Assays designed by
relax mode and have no
off-target hits

One primer sits in the
repeat region

Two primers sits in the
repeat region

One primer sits in the
repeat region

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Two primers sits in the
repeat region

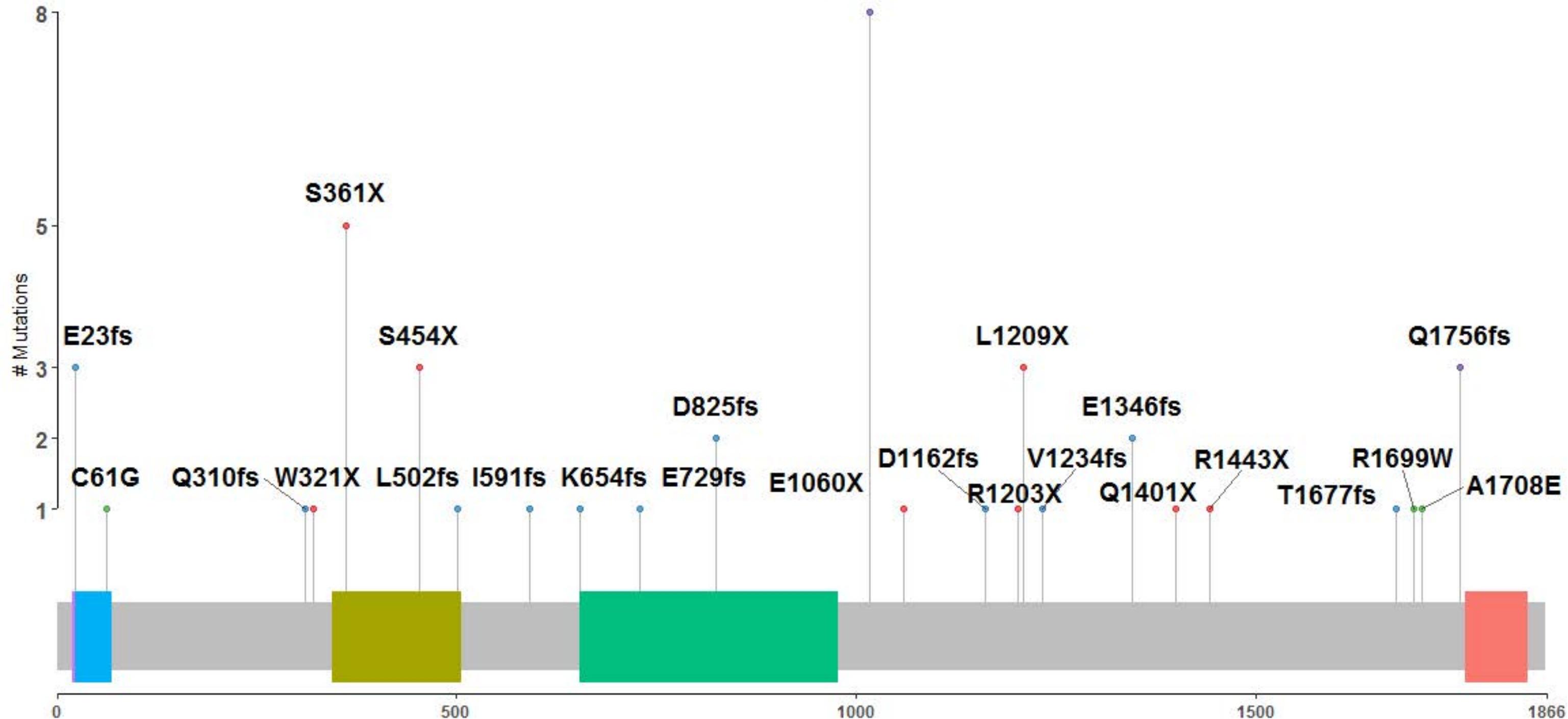
Two primers sits in the
repeat region

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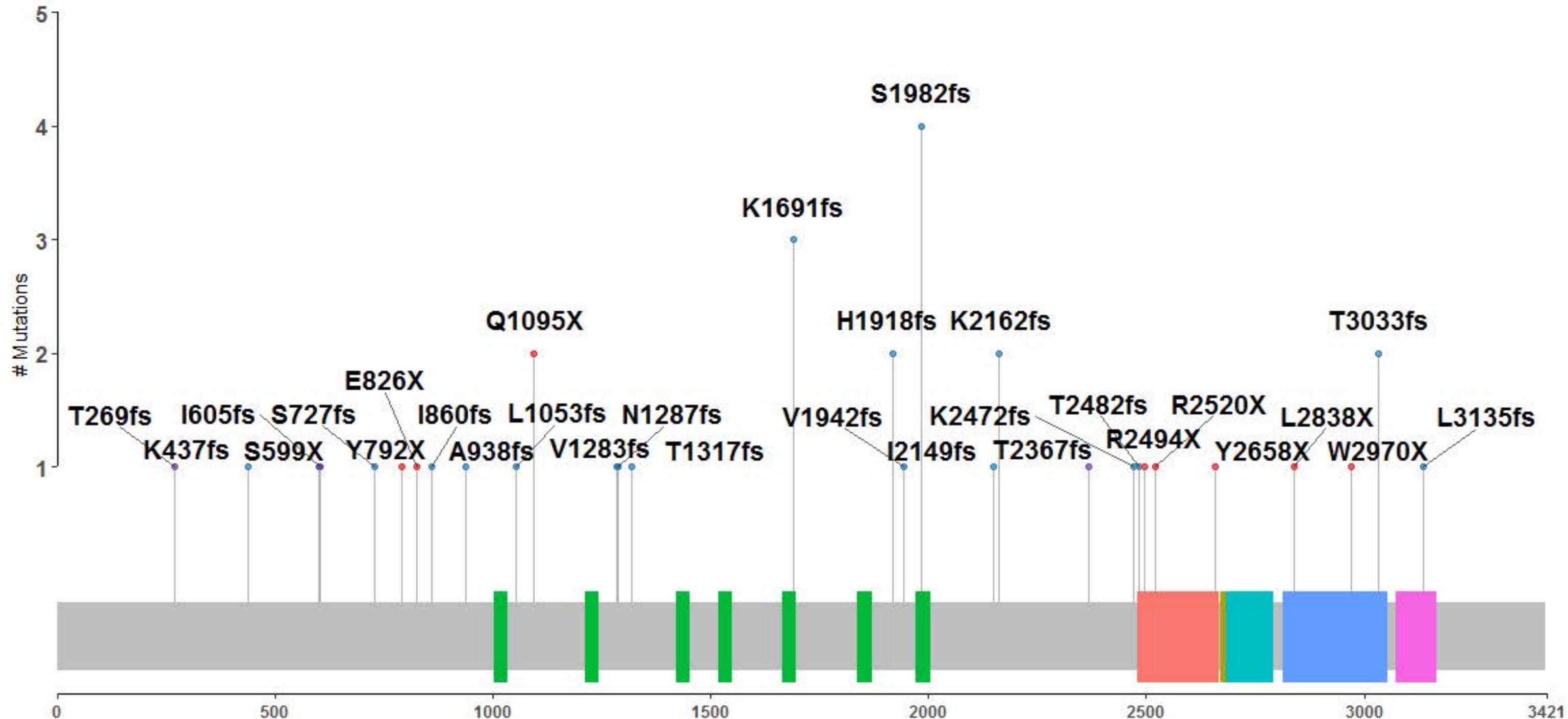
BRCA2	BRCA2_3_UTR_10	13	32973633	32973831	ACACTGACGACATGGTTCTA CATGTAACCTAATTCCCTTT TACTATTCAGT	TACGGTAGCAGAGACTTGGTCTG CGCTAAAAATAAGCAGGCAGA	199	28	TGTAACTCTAATTCCCTTTACTATTCCAGTGTGATCTCTGAAATTAAATT ACTTCAACTAAAATTCAAACTTTAACATCAGAAGATTCTAGTAAATT ATTTTTTTTCAACAAAATGGTCATCCAACACTCAAACCTGAGAAAATATC TTGCTTCAATTGGCACTGATTCTGCCGCTTTATTTCAGCG TCAACAAAATGGTACATCCAACACTCAAACCTGAGAAAATATCTTGCTTCAA ATTGGCACTGATTCTGCCGCTTTATTTCAGCGTATCACAGGACCCAG AGCCTATGCCCTTTAAACTTACCAAAAAGCAGAAAGATTAACTCAATTAA AGATGATACTCTATTGTTACGTCCTtttt CTGAGCTCGGTGGCTCATGCCGCTTAATCCAACACTTGAGAAGCTGAG GTGGGAGGAGTGCTTGAGGCCAGGAGTCAAGACCACCTGGCCAACA TAGGGGAGACCCCACATTTACAAAGAAAAAAAAGGGAAAAGAAAAT CTTT CTGCGAGGAAGACAGGTGATCCGAATCTTAAAGTGCAAAAGATGGGC CGGGTGTGGTGGCTCATGCCGCTTAATCCACCGCCTTGGAGGGCCAG GCAGGCAGATCACCTGAGGTGGAGGGTGAAGACCAACTGACCAACA ACGGAGAAAACCCGCTCTACTTTAAAATGCAAGTGGCGTGC AGACTGACCAACAAACGGAGAAACCCGCTCTACTTTAAAATGCAAGT TAGCGTGCCTGGTGGCCCATGCCGTATTCCAGCTACTCGGGAGGC TGAGGCAGGAGAACCACTGATCCCTGGAGGCCAGTGGCGTGAGC GGAGATTGCGCCATTGACACCAGCCGGCCACAAGAGCGAAACTCC GTCTCA GTTGGGTGAGCGGAGATGGCCATTGCCACACCAGCCGGGCCACAA GAGCGAAAACCTCGTCTCAAAAAAAAAAGCAAAAGATACTACCAAGGCC GCGGAGCAAGGTACCTCACACTTGTGAGCGAGTTAAGATGGGTTCAC AATTTCAGCAAGGAAACGG
BRCA2	BRCA2_3_UTR_11	13	32973746	32973929	ACACTGACGACATGGTTCTA CATCAACAAAATGGTACATCC AAACTCAA	TACGGTAGCAGAGACTTGGTCTA aaaaaaGGACGTACAAATGAGAGT AT	184	35	One primer sits in the repeat region
BRCA2_3_UTR	BRCA2_3_UTR_12	13	32973158	32973307	ACACTGACGACATGGTTCTA CACTGAGCTCGGTGGCTCAT	TACGGTAGCAGAGACTTGGTCTA AAGATTTCCTTCCCCTTTT	150	49	
BRCA2_Pro_moter_Combined	BRCA2_Pro_moter_Com_bined_14	13	32888661	32888849	ACACTGACGACATGGTTCTA CACTGCGAGGAAGACAGGT GAT	TACGGTAGCAGAGACTTGGTCTG CACGGCTACTTCGATT	189	55	
BRCA2_Pro_moter_Combined	BRCA2_Pro_moter_Com_bined_15	13	32888792	32888990	ACACTGACGACATGGTTCTA CAAGACTGACCAACACGGA GAA	TACGGTAGCAGAGACTTGGTCTT GAGACGGAGTTCGCTTT	199	57	
BRCA2_Pro_moter_Combined	BRCA2_Pro_moter_Com_bined_16	13	32888925	32889092	ACACTGACGACATGGTTCTA CAGTTGCGGTGAGCGGAGA T	TACGGTAGCAGAGACTTGGTCTC CGTTTCTGCTTAAAAAA	168	50	

N1018fs

- Frame_Shift_Del
- Frame_Shift_Ins
- Missense_Mutation

- Nonsense_Mutation

- BRCT
- BRCT_assoc
- EIN3
- RING
- zf-C3HC4_2



- Frame_Shift_Del
- Frame_Shift_Ins
- Nonsense_Mutation

- BRCA-2_helical
- BRCA-2_OB1
- BRCA2
- BRCA2DBD_OB1
- BRCA2DBD_OB2
- BRCA2DBD_OB3

Unselected-detected

Clinically-tested

